Binaurality and azimuth tuning of neurons in the auditory cortex of the big brown bat

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Abstract **By using a combined closed and free-field stimulation system, binaurality and azimuth tuning of the neurons in the auditory cortex of the big brown bat, *Eptesicus fuscus*, were studied. A variety of azimuth-tuning functions were demonstrated for the binaural neurons. The large majority of EE (contralateral and ipsilateral excitatory) neurons exhibited azimuth selectivity with the best azimuths (BA) at contralateral 30°—40°, some at ipsilateral 20°—40° and preferred azimuth ranges (PAR, response amplitude ≥75% of maximum) between 8° and 40°. Sound source azimuths strongly modulate spike counts with a mean modulation depth of 83.8% for EE neurons. EI (contralateral excitatory and ipsilateral inhibitory) neurons have simple azimuth tuning with BA located at contralateral 20°—40° and a broad PAR ranged from 30° to 55°. The present results suggest that azimuth-tuning characteristics of binaural neurons in the auditory cortex of the bat are of significance for acoustic behaviour.**

Keywords: bat, auditory cortex, binaural neurons, azimuth tuning.

How the brain solves the scene analysis problem in hearing is not known[1]. Among various elements forming auditory maps, the location of a sound source in space is an important one. Behavioral studies imply that auditory cortex plays a pivotal role in spatial hearing[2]. However, there is a big controversy over neural mechanisms of auditory localization in mammals, but little evidence shows that spatial information is encoded by either the topographical or distributed code in the auditory cortex[3,4]. Physiological studies have demonstrated that many "high directional" neurons in the primary auditory cortex of cat[5—8] and monkey[9] respond strongly to sounds presented across areas as large as half the sound field. Moreover, the spatial tuning of most neurons broadens considerably as the stimulus intensity is increased to more than ~20 dB above the neuron's threshold. Consequently, these cortical neurons are not able to play a role in the accurate sound localization.

Behavioral studies indicate that echolocating bats appear able to determine the direction of an echo's source within ±2°—5°[10]. Results show that binaural cortical neurons have directionality[11] and functional organizations[12]. Manabe et al.[11] found that the binaural EI (contralateral excitatory and ipsilateral inhibitory) cortical neurons are directionally sensitive, while the EE (contralateral and ipsilateral excitatory) neurons are not in the mustache bat, *Pteronotus parnell*. The fact that bats have excellent ability in echolocation implicates that the bat must have evolved specific auditory neurons or neural mechanisms for the computation of spatial information.

The present note studied the binaurality and azimuth tuning characteristics of individual neurons in the auditory cortex by using a combined closed and free-field stimulation system, and tried to reveal their possible strategy in spatial coding.

1 Materials and methods

(i) Materials. 4 female or male big brown bats, *Eptesicus fuscus*, (body weight 17.5—22.5 g) with normal hearing were selected for recording sound-activated responses from cortical neurons. The procedures were the same as in previous study[12]. Briefly, anesthesia was induced with Nembutal (45—50 mg/kg b.w.) for surgery. During recording, each bat was administered neuroleptanalgesic Innovar-Vet (0.08 mg/kg b.w. of fentanyl, 4 mg/kg b.w. of droperidol) to keep the animal hemi-awake. The steel post glued on the bat's skull was fixed on a metal rod with a set screw for stabilizing the head. The experiments were conducted with the approval of the Institutional Animal Care and Use Committee (#1438) of the University of Missouri-Columbia.

(ii) Recording. Recordings were made with 3 mol • L^{-1} KCl-filled glass electrodes (impedance: 10—15 MΩ) from penetrations through small holes in the skull above the primary auditory cortex (A1). Action potentials from individual neurons were fed to a computer for acquisition of post-stimulus-time histogram (PSTH) and dot displays of the neuron's responses over 20 trials at 1 pulse per second. The total number of impulses, frequency tuning curve and temporal discharge pattern of each neuron were obtained from the PSTH and used to quantify a neuron's response to these stimuli.

(iii) Acoustic stimuli. The closed stimulus delivery system consisted of two independent 1/4-inch Brüel & Kjaer (4135) microphones and generated two tonal stimuli (4 ms, 0.5-ms rise-decay times) through a custom-made plastic adaptor with its tip inserted into the funnels of the external ears. Sound intensities were calibrated with a 1/8-inch B&K (4138) microphone placed at the mouth of the adaptor tip and expressed in dB SPL, 0 dB referred to 20 μPa. The free-field system for the analysis of azimuth sensitivity consisted of a loudspeaker presenting tone bursts. The loudspeaker was attached to a 20-cm-long aluminum arm, moved horizontally around the bat's head equidistantly to any specific position in the frontal hemi-field (± 80° from the midline, 0° elevation) in 20° or 10°-steps, remotely controlled by two servomotors, and monitored by a calibrated oscilloscope outside the sound-proof and anechoic room.

The binaurality of the neurons was determined by a method of interaural intensity difference (IID), which kept the intensity of the stimuli at the contralateral ear constant (20 dB above threshold), generally ⩾65 dB SPL, varying the level at the ipsilateral ear alone. Tone bursts were presented to each ear separately. Frequency and IID were systematically changed to measure the neuron's characteristic frequency (CF), minimum threshold (MT), frequency tuning curve, and response latency at 20 dB above threshold. Under free-field sound stimuli, response amplitudes of individual neurons expressed in a total of spike counts were measured when the azimuth of sounds at CF and 20 dB above threshold was randomly changed.

(iv) Data analysis. The binaurality of the neuron was judged based on responses to sound stimuli presented to contralateral or ipsilateral ear. The IID functions of EE or EI neurons were constructed with spike counts by 20 stimuli and latency at 20 dB above threshold as the ordinate, and IID as abscissa. The azimuth tuning curves were drawn with spike counts as the ordinate and azimuth as the abscissa. The azimuth to which the neuron is most sensitive with a maximum of spikes was referred as "best azimuth" (BA), and the azimuth range over which responsiveness was ⩾75% of maximum as "preferred azimuth range" (PAR). The azimuth tuning characteristics for EE or EI neurons were classified on the basis of BA and PAR. The depth of modulation of spike counts by azimuth was given as the maximum percentage reduction of spike count as a constant-level sound source was varied in location through 180°, i.e. 100 × (1–min/max), where min and max were the minimum and maximum of spike counts within the azimuth range investigated.

2 Results and discussion

(i) Binaurality of auditory cortical neurons. At the beginning of experiments, acoustic stimuli were separately presented by a closed stimulus delivery system to two ears in order to identify binaurality of individual neurons. As shown in fig. 1, EE neurons received excitatory inputs from both ears and fired impulses under contra- or ipsilateral sounds. EI neurons received excitatory inputs from contralateral ear and inhibitory inputs from ipsilateral ear. It is noticeable that spike counts of EE neurons evoked by sound stimulation to both ears with the same frequency and intensity (IID = 0) were more than the sum of spike counts evoked separately from each ear by the same sound. This implies that notable non-linear positive or negative gains occurred during the integration of inputs from two ears by binaural cortical neurons. The size of gains depends upon the characters of different neurons.

A total of 61 EE and 84 EI neurons were ascertained. The CF of EE neurons ranged from 40.3 to 67 kHz, MT from 32 to 78 dB SPL (mean ± S.D., the same below, 47.3 ± 10.3 dB SPL) at contralateral stimulation, MT from 38 to 95 dB SPL (62.5 ± 12.8 dB SPL) at ipsilateral stimulation, and latency from 7 to 26.5 ms (14.7 ± 3.7 ms) at contralateral stimulation and from 7.5 to 35 ms (16.4 ± 6.1 ms) at ipsilateral stimulation. The MT and latency of EE neurons at ipsilateral stimulation were significantly more than those at contralateral stimulation. The CF of EI neurons ranged from 40.1 to 78 kHz, MT from 36 to 78 dB SPL (48.3 ± 9.7 dB SPL), and latency from 9 to 19.5 ms (14.6 ± 2.9 ms) at contralateral stimulation. It appears that the differences in MT and latency between EE and EI neurons are very small at contralateral stimulation.

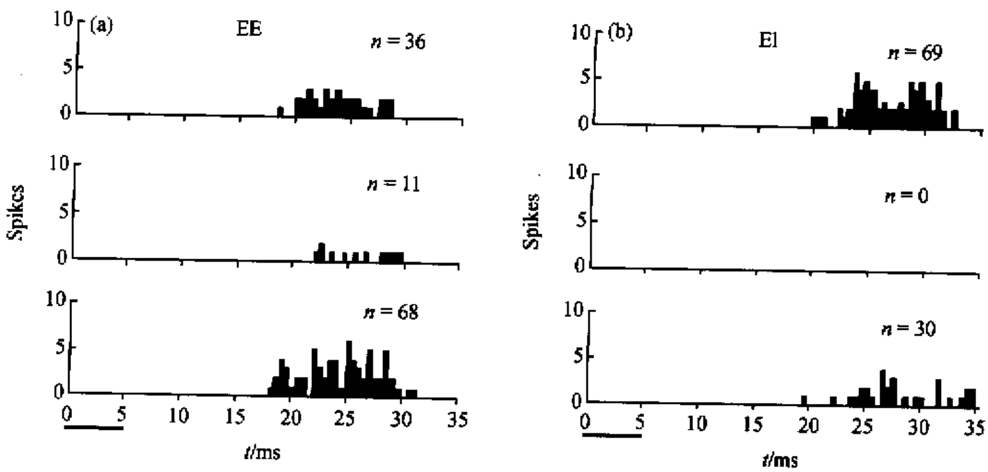

Fig. 1. Responses of single EE (a) and EI (b) neuron in the auditory cortex of the big brown bat to different stimuli in PSTH. *n*, Spike counts evoked by 20 stimuli; bar, acoustic stimulus. Top histogram, By contralateral stimulus alone; middle, by ipsilateral stimulus alone; bottom, by bilateral stimuli, IID = 0.

Response properties of binaural cortical neurons are obviously correlated with IID. Fig. 2 illustrates IID functions for typical EE and EI neurons. When IID was increased from −20 to 20 dB, spike count of the EE neuron evoked by 20 stimuli increased from 24 to 113, and latency decreased from 10.6 to 8.2 ms. In comparison, spike count of the EI neuron gradually decreased from 85 to 27, and latency increased from 16 to 20.5 ms. These IID functions show that EE and EI neurons are able to encode IID through spike count or latency shift. As IIDs *per se* reflect azimuth information of a sound source, the binaural cortical neurons must play an important role in sound localization.

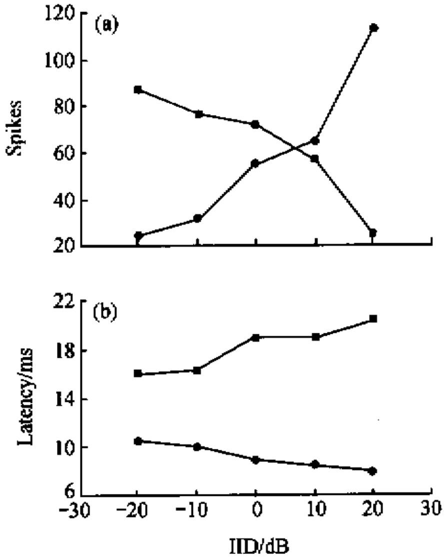

Fig. 2. Spike count (a) and latency (b) of a single EE (●) and EI (■) neuron in the auditory cortex of the big brown bat as a function of interaural intensity difference (IID).

(ii) Azimuth tuning of EE cortical neurons. After the binaurality of the neurons in the auditory cortex was determined, responses of those neurons to varying azimuth were investigated by using a free-field stimulation system. The azimuth tuning characteristics of 12 EE neurons were individually demonstrated. On the basis of spike count vs azimuth functions, the location of BA and the extent of PAR, EE neurons were categorized into 4 classes: contralateral-max (7/12, 58%), ipsilateral-max (2/12, 17%), dual-peaked (2/12, 17%), and omnidirectional (1/12; 8%), as shown in fig. 3 (a—d) respectively.

The locations of BA of most contralateral-max EE neurons were at contralateral 30°, and some at ipsilateral

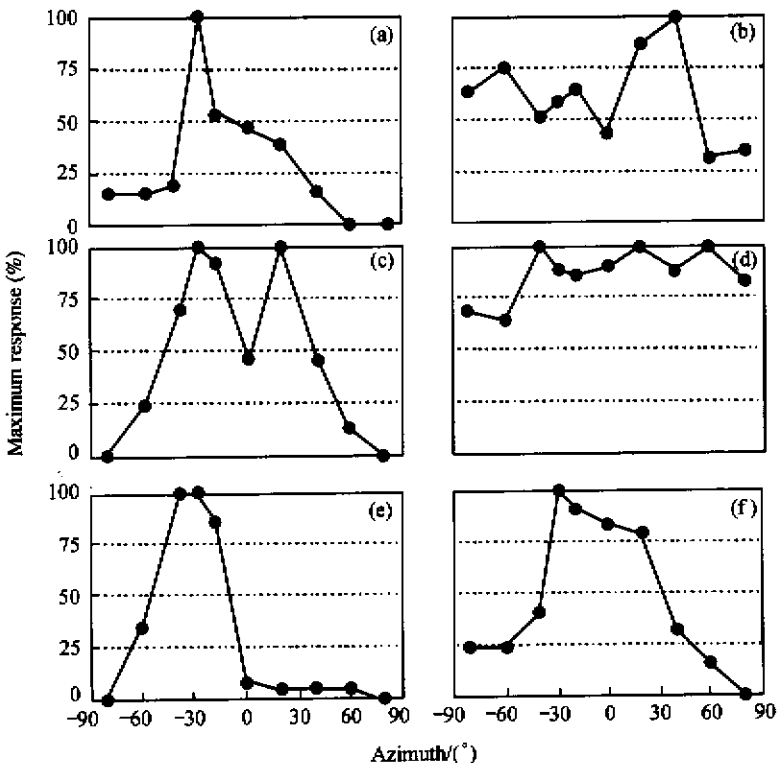

Fig. 3. Azimuth tuning of EE (a – d) and EI (e, f) neurons in the auditory cortex of the big brown bat. Response amplitude at each location of azimuth expressed in % of maximal response. 0°, midline in front of animal head; negative on the left of 0°, contralateral azimuths, positive on the right of 0°, ipsilateral azimuths. ID number, CF, maximum of spike count per stimulus, and azimuth tuning type for each neuron are in turn as follows: (a) #710-2, 61.2 kHz, 5.6, contralateral-max; (b) #727-1, 47.9 kHz, 1.0, ipsilateral-max; (c) #321-3, 66.8 kHz, 2.7, dual-peaked; (d) #321-5, 67.0 kHz, 0.7, omnidirectional; (e) #316-3, 41.0 kHz, 4.4, contralateral-max; (f) #710-4, 78.3 kHz, 6.0, antero-contralateral preferring. Preferred azimuth range (PAR), responses ≥ 75% of maximum.

20° or 40°. They were sharply tuned to azimuth with a narrow PAR ranged from 8° to 26°, only few PAR of about 40°. The BA of ipsilateral-max EE neurons was located at ipsilateral 30° or 20° with a little broad PAR from 34° to 66°. The BAs of dual-peaked EE neurons were located at contralateral 30° and ipsilateral 20°, respectively, with a narrow PAR ranged from 18° to 26°. The omnidirectional EE neuron had multiple BA locations at contralateral 40°, ipsilateral 20° and 60° with a very broad PAR of about 140°, encompassing much of the frontal sound field.

We quantified the azimuth sensitivity of neurons by computing the depth of modulation of spike counts by azimuth. The mean modulation depth for contralateral-max EE neurons was 95%, 80% for ipsilateral-max, and 94% for dual-peaked, respectively. Statistically, 83.3% of EE neurons showed ≥75% modulation of spike counts with the median depth of 83.8%. It suggests that most of cortical EE neurons were high directionally sensitive.

It has long been recognized that EE binaural neurons in the auditory cortex of mammals indicated only "there is a sound or not" and were not directionally sensitive. These neurons could not provide available information for detection of a sound source direction as their azimuth tuning is similar to that of ominiderectional EE neuron above-mentioned[11], or they show sharply azimuth tuning only at the intensity of about threshold[5—9]. In contrast, the present results demonstrate that a large proportion of EE neurons in the auditory cortex of the bat show quite sharp azimuth tuning functions and different types with BA located at contralateral 30°, or ipsilateral 20° or 40° as well, even at the intensity ≥65 dB SPL. The preferred azimuth characteristics of these cortical neurons can not be explained by the amplification of the bat's auricle[13, 14], but the ipsilateral facilitation may be involved[15]. It appears that sharply azimuth-tuned EE neurons in the auditory cortex of the bat not only detect sound sensitively but also play an important role in the accurate localization of a sound source.

(iii) Azimuth tuning of EI cortical neurons. Responses of 24 cortical EI neurons in the bat to sounds at CF and 20 dB above threshold presented at different azimuths were measured. It was found that azimuth-tuning functions for EI neurons were simpler than those for EE neurons. As fig. 3(e, f) show, there are two types: contralateral-max (13/24; 54.2%) and antero-contralateral preferring (11/24; 45.8%). Their BAs were located at contralateral 20°—40°, and PARs were significantly distinct: contralateral-max PARs limited only the contralateral site, not across the midline and were quite narrow (42.1° ± 21.8°); antero-contralateral preferred PARs located at both sides across the midline and were rather broad (61.4° ± 25.6°). The modulation depths by azimuth for EI neurons were close to 100%, showing good directionality. However, broaden PARs of EI neurons implicate that their azimuth tuning is not sharp enough. It appears that during the bat's echolocation, EI neurons function as the detecting rough location of a sound source and EE neurons of sharp azimuth tuning must participate in accurate sound localization. In conclusion, the present results demonstrate that echolocating bats have evolved sharply azimuth-tuned neurons in the auditory cortex, distinctly different from other mammals. It must be of great importance for successfully intercepting prey and avoiding obstacles in the bat.

Acknowledgements This work was conducted in the laboratory of P. Jen of University of Missouri-Columbia, MO and was supported by a grant from the Human Frontier Science Program. The preparation of this manuscript is supported by the National Natural Science Foundation of China (Grant Nos. 39470203 and 39770209).

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(Received January 18, 2002)