Auditory cortical neurons in the mouse have salient best azimuths

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Azimuth sensitivity of the primary auditory cortex in mammals is not well understood, and there is a debate as to whether the primary auditory cortex plays role in spatial hearing. We show for the first time, using single-unit recordings in the mouse primary auditory cortex, that auditory cortical neurons demonstrate a variety of azimuth-tuning functions and there are a majority of neurons showing salient best azimuths. The findings differ from those in the cat, ferret and monkey, and imply that there is some representation of auditory space in the mouse primary auditory cortex. *NeuroReport* 16:2007–2010 © 2005 Lippincott Williams & Wilkins.

Keywords: auditory cortex, azimuth sensitivity, best azimuth, characteristic frequency, preferred azimuth range, single-unit recording

Introduction

An intact auditory cortex is essential for normal sound-localization behavior. Unilateral lesions of auditory cortex result in contralateral deficits in sound localization [1]. A number of auditory cortex studies have failed to demonstrate any evidence of narrow tuned neurons as the receptive fields of many cortical neurons encompass a hemifield or more of auditory space and broaden further with increases of stimulus intensity in mammalian species (e.g. cat) [2]. Also, there is the evidence for obvious qualitative differences in spatial sensitivity within the primary auditory cortex (A1) in the cat [3], ferret [4] and big brown bat [5]. Thus, there is debate as to whether the A1 plays a distinct role in spatial hearing.

A tonotopy that largely reflects the gradient of cochlear frequency representation has been well known in the auditory cortex of mammals. Neurons of similar characteristic frequency (CF; the frequency to which the neuron had the lowest threshold) are organized in isofrequency contours within some auditory cortex areas (see review [6]), and as has been demonstrated in the mouse [7,8]. The relationship between tonotopy and spatial sensitivity within the auditory cortex, however, is also not clear yet [3,9–11].

To examine this issue, we used extracellular recording via glass microelectrodes to determine the responses of single cortical neurons of the BALB/c mouse to sounds and evaluated their azimuth sensitivity.

Materials and methods

Animal preparation

A total of 24 healthy female BALB/c strain mice (4–6 weeks), weighing 12–15 g, with normal hearing were used. All procedures were approved by the Institute of Biophysics Committee on Use and Care of Animals. Atropine sulfate (0.05 mg/kg body weight) was given prophylactically at the beginning of surgery to minimize respiratory congestion. Mice were initially sedated with pentobarbital sodium (60 mg/kg body weight) intraperitoneally. A surgical level of anesthesia sufficient to suppress the withdrawal reflex was maintained by continuous subcutaneous infusion of pentobarbital sodium (8 mg/kg body weight) throughout the duration of an experiment. At the end of the experiment, the animal was given a lethal dose of anesthetic.

Surgery

Once the mouse was anesthetized, the midline incision was made in the scalp. A portion of the scalp and underlying temporalis muscle was removed from the left side. The flat head of a stainless steel screw was attached over midline of the exposed skull with dental acrylic adhesive. An opening of about 2 mm in diameter was created in the skull using a dental bur, and the dura that covered the left primary auditory cortex was exposed. The animal was supported in a frame, its body resting on a plane shelf, and its head rigidly fixed by clamping the head-support post to the frame; the head was oriented forward and horizontally with respect to the horizontal plane parallel to the floor.

Acoustic stimuli

Stimuli were presented under free-field conditions. The mouse was located in a shielded, soundproof anechoic chamber. The ambient sound level in the chamber was 34 dB sound pressure level (A-weighted). The loudspeaker (ES1, Tucker-Davis Technologies, Alachua, Florida, USA;

frequency range 2–110 kHz) was located 50 cm away and was horizontally moveable around the head of the mouse. The location directly ahead of the animal was designated as 0° azimuth, 0° elevation. The loudspeaker was moved randomly between contralateral 90° and ipsilateral 90° (in 30° steps); azimuths to the right of the animal were assigned negative values, and those to the left, positive values.

Pure-tone bursts of 50 ms duration with rise and fall times of 5 ms each were produced by Tucker-Davis Technologies-3 System and delivered at a rate of one per 3 s. The frequency ranged from 5 to 45 kHz. Sound intensities were determined

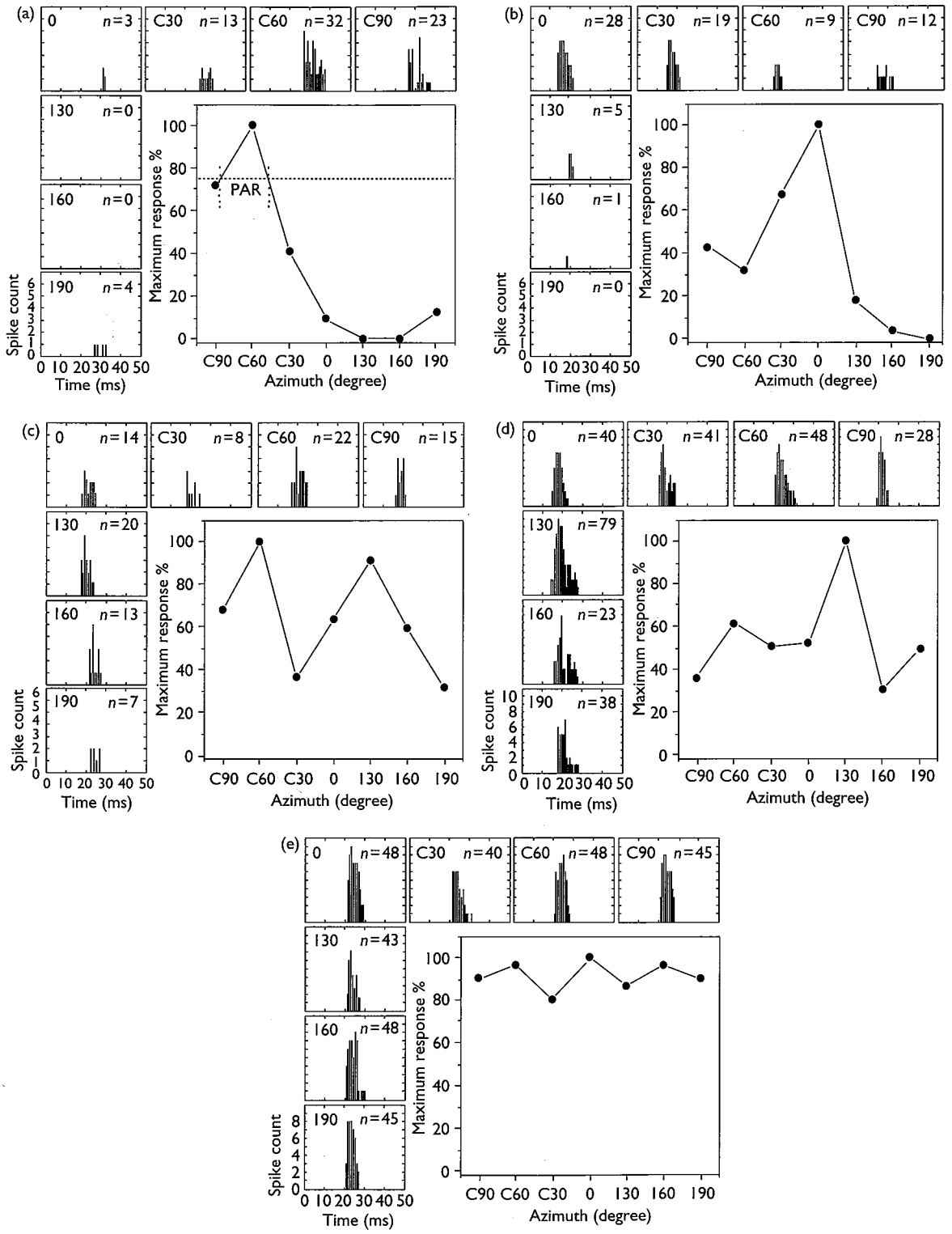

Fig. 1 Azimuth-tuning functions of auditory cortical neurons in the mouse primary auditory cortex (AI) based on spike count: (a) contralateral-max, (b) middle line, (c) dual-peaked, (d) ipsilateral-max and (e) omnidirectional. *n*=number of spikes.

at the animal's pinna with a Brüel and Kjaer 4135 microphone (Naerum, Denmark) and measuring amplifier (B&K Type 2610) in dB sound pressure level (0 dB sound pressure level re. 20 μPa).

Recording sessions

Glass microelectrodes filled with 3 M sodium acetate (impedances 10–15 MΩ) were orthogonally inserted into the surface of the auditory cortex by a remote-controlled Pulse Motor Micro-Drive Micromanipulator (SM-21, Narishige, Tokyo, Japan) with an accuracy of 1 μm, and neurons identified along the penetration track by their response to sound. An indifferent electrode was placed at the nearby temporal muscles. Action potentials of single neurons were recorded by Tucker-Davis Technologies-3 System.

Data analysis

Receptive field properties such as CF, minimum threshold, response latency at an intensity 20 dB above minimum threshold and spatial tuning were determined by systematically adjusting stimulus intensity, frequency and azimuth.

A maximum response of spike counts of each neuron activated by sound source at various azimuths was calculated. The azimuth sensitivity of each neuron was characterized on the basis of two measures: best azimuth and preferred azimuth range. Best azimuth was defined as the sound azimuth at which the neuron fired with a maximum of spike count. The preferred azimuth range was defined as the azimuth range throughout which normalized response amplitude (spikes per stimulus) should be approximately 75% of maximum.

Results

A total of 323 neurons were recorded from the A1 of 24 mice. Most of the neurons fired tonically (about 78.3%), others phasically (21.7%) in response to tone bursts of 50 ms duration. The CF of recorded neurons ranged from 10 to 24 kHz, and 94.7% neurons had CFs between 12 and 23 kHz.

On the basis of the measures of spike counts at a higher sound level (the neuron threshold plus 20 dB) to CF tones we determined azimuth-tuning curves as a function of sound azimuths for each isolated neuron. Five types of azimuth-tuning functions (Fig. 1a–e) could be categorized according to the location of the response maximum: contralateral-max (a), 231/323 (about 71%); single peak at the midline (b), 11/323 (3%); two-peaked with higher peak at the contralateral side (c), 64/323 (20%); ipsilateral-max (d), 2/323 (<1%) and omnidirectional (e), 15/323 (<5%), whose response amplitudes at all azimuths were more than 75% of the maximum. More than 90% of cortical neurons were remarkably sensitive to sounds located in the contralateral hemifield, that is, represented by contralateral-max and dual-peaked response types.

The peak of the azimuth-tuning curve is defined as the best azimuth of the neuron. Most of the recorded neurons had salient best azimuth, the location at which the neuron fired with a maximum of spike counts. About 91% (295/323) neurons had best azimuth at the contralateral side, about 3% (11/323) at the midline (0° azimuth) and <1% (2/323) at the ipsilateral side, whereas omnidirectional neurons (<5%, 15/323) had no best azimuth. Figure 2a illustrates that the distribution of contralateral locations of 90° (C90°), 60° (C60°) and 30° (C30°) were about 29% (93/323), 47% (151/323) and 16% (51/323) of neurons, respectively. Two neurons had best azimuth at ipsilateral 30° (I30°) or 90° (I90°).

The preferred azimuth range is defined as the range of azimuths over which the response (spikes per stimulus) was ≥75% of maximum [3]. Calculated from the azimuth-tuning function, the preferred azimuth range of all 323 neurons ranged from 11° to 180°. A neuron with a preferred azimuth range of less than or equal to 60° was designated as *sharply azimuth tuned*. We found about 11% of all recorded neurons of preferred azimuth range of less than or equal to 30° and 37% between 30° and 60°. Thus, nearly half of the azimuth-sensitive neurons were sharply azimuth tuned. Only 6% of the neurons had a preferred azimuth range of more than 120°.

Another important aspect was that the azimuth-sensitive neurons had various CFs within the range of 10–24 kHz, as

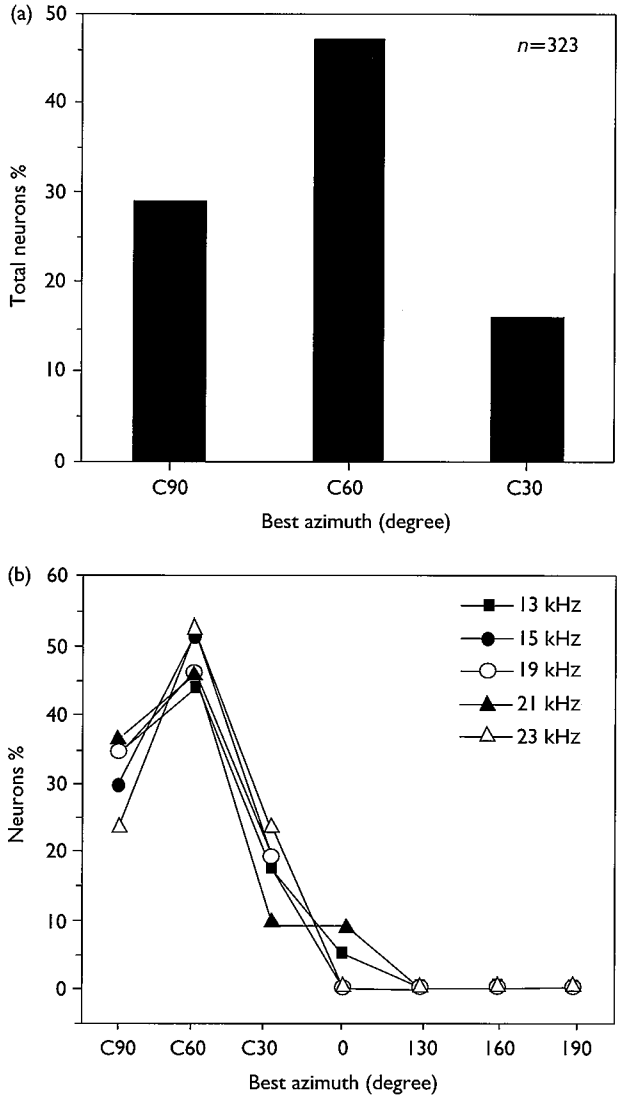

Fig. 2 Best azimuth and characteristic frequency (CF) of auditory cortical neurons in the mouse primary auditory cortex (AI). (a) Distribution of the best azimuths for auditory cortical neurons in the mouse AI at contralateral 90° (C90), 60° (C60) and 30° (C30), respectively. *n*=total number of neurons. (b) Proportion of cells responding best to each azimuth at five selected CFs.

examined in this study. As shown in Fig. 2a, for each given best azimuth (e.g. C90°, C60°, C30° or 0°), there were many best azimuth-tuned neurons, whose CFs spanned over the range from 10 to 24 kHz. Figure 2b illustrates the proportions of cells responding best to each azimuth at five selected CFs. The curves calculated at other CFs were very similar to the abovementioned functions. All the functions were nearly the same, indicating that the distribution of auditory cortical cells in the mouse A1 at each best azimuth was independent of CF.

Discussion and conclusions

In comparison with similar studies in other mammals, there are significant differences between the proportions of A1 neurons showing the different types of azimuthal sensitivity in the mouse or big brown bat [5] and those obtained in the cat [10,12], ferret [4] and monkey [13]. In the present study, contralateral-max and two-peaked types in the mouse constituted about 91% of all responses, much more than 45.9% in the cat [10]. Much fewer omnidirectional units were obtained in our study (<5% to testing with CF tones) than the 19.9% obtained in cats [10].

The fact that there were different proportions of the mouse A1 neurons with various best azimuths (e.g. at C90°, C60°, C30° or 0°) may imply that the cortical neurons do have distinct preferred sound-source location at which the neurons could be most responsive when evoked by CF stimuli. This may be consistent with the notion that they were produced by the pinna's acoustic axis, as the acoustic axis of the mouse pinna is presumably around contralateral 30–40° in azimuth. Why the proportions of best azimuths at contralateral 90° or 60° were higher in the mouse A1, however, is still not clear.

With regard to azimuth function–CF relationship for cat A1 neurons, two studies [2,10] reported that, in general, there were no marked differences between the types of azimuth function in the two different CF ranges (CFs $\leq$ 12 kHz and CFs $\geq$ 12 kHz). We analyzed our data on best azimuth versus CF for all recorded A1 neurons and then deduced a consistent conclusion that the best azimuth of A1 neurons may be independent of their CF. This fact accords with spatial localization of a sound source for normal mammals.

References

1. Jenkins WM, Merzenich MM. Role of cat primary auditory cortex for sound-localization behavior. *J Neurophysiol* 1984; **52**:819–847.
2. Middlebrooks JC, Pettigrew JD. Functional classes of neurons in primary auditory cortex of the cat distinguished by sensitivity to sound location. *J Neurosci* 1981; **1**:107–120.
3. Clarey JC, Barone P, Imig TJ. Functional organization of sound direction and sound pressure level in primary auditory cortex of the cat. *J Neurophysiol* 1994; **72**:2383–2405.
4. Mrsic-Flogel TD, King AJ, Schnupp JWH. Encoding of virtual acoustic space stimuli by neurons in ferret primary auditory cortex. *J Neurophysiol* 2005; **93**:3489–3503.
5. Shen JX, Chen QC. Binaurality and azimuth tuning of neurons in the auditory cortex of the big brown bat. *Chin Sci Bull* 2002; **47**:1024–1027.
6. Ehret G. The auditory cortex. *J Comp Physiol A* 1997; **181**:547–557.
7. Stiebler I, Neulist R, Fichtel I, Ehret G. The auditory cortex of the house mouse: left–right differences, tonotopic organization and quantitative analysis of frequency representation. *J Comp Physiol* 1997; **181**:559–571.
8. Shen JX, Xu ZM, Yao YD. Topography of acoustic response characteristics in the auditory cortex of the Kunming mouse. *Chin Sci Bull* 2000; **45**:443–448.
9. Suga N, Jen PHS. Disproportionate tonotopic representation for processing species-specific CF-FM sonar signals in the mustache bat auditory cortex. *Science* 1976; **194**:542–544.
10. Rajan R, Aitkin LM, Irvine DRF, McKay J. Azimuthal sensitivity of neurons in primary auditory cortex of cats. I. Types of sensitivity and the effects of variations in stimulus parameters. *J Neurophysiol* 1990; **64**:872–887.
11. Shen JX, Chen QC, Jen PHS. Binaural and frequency representation in the primary auditory cortex of the big brown bat, *Eptesicus fuscus*. *J Comp Physiol A* 1997; **181**:591–597.
12. Imig TJ, Irons WA, Samson FR. Single-unit selectivity to azimuthal direction and sound pressure level of noise bursts in cat high-frequency primary auditory cortex. *J Neurophysiol* 1990; **63**:1448–1466.
13. Ahissar M, Ahissar E, Bergman H, Vaadia E. Encoding of sound-source location and movement: activity of single neurons and interactions between adjacent neurons in the monkey auditory cortex. *J Neurophysiol* 1992; **67**:203–215.