

Columnar and layer-specific representation of spatial sensitivity in mouse primary auditory cortex

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The primary auditory cortex (AI) is implicated in coding sound location, as revealed by behavior-lesion experiments, but our knowledge about the functional organization and laminar specificity of neural spatial sensitivity is still very limited. Using single-unit recordings in mouse AI, we show that (i) an inverse relationship between onset latency and spike count is consistently observed when all the azimuthal points are taken; (ii) a substantial proportion of penetrations perpendicular to the AI surface showed columnar organization of best azimuths; (iii) the preferred azimuth range of AI neurons demonstrated layer-specific distribution pattern. Our findings suggest that similar to other response properties, the manner of sound space information processing in the auditory cortex is also layer dependent. *NeuroReport*

Introduction

Lesion and pharmacological inactivation studies have shown that the primary auditory cortex (AI) plays an essential role in sound-localization behavior [1–3]. Various degrees of sensitivity of AI neurons to sound–source direction have been shown by electrophysiological studies in cat [4], ferret [5], and monkey [6]. It has been suggested that not only the variations in spike count, but also the timing of spikes can be used to characterize the spatial sensitivity of neurons in AI [7] or other cortical areas [8,9]. Nowadays, mouse is becoming a popular animal model for physiological and behavioral studies, but is still not commonly studied yet in the auditory cortex. In addition to our previous study [10], data on the azimuth tuning properties of mice AI neurons, and how spike timing, and spike count are associated during sound–source localization is still very limited.

Evidence in support of a precise anatomical lamination of auditory cortex is compelling [11]. Each layer differs in many aspects, such as its neuronal architecture and cytoarchitecture, gamma-aminobutyric acidergic organization and thalamic input, thus resulting in systematic laminar differences in auditory receptive field properties, such as response latency, frequency tuning bandwidth, intensity thresholds, and temporal and spectral modulation transfer functions [12–15]. However, whether the property of spatial selectivity also varies systematically across different layers of the auditory cortex has only been poorly studied. In addition, columnar organization of characteristic frequency [16], intensity threshold [17], frequency sweep tuning [18], and binaural response properties [19] have been reported in the auditory cortex.

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However, it still needs to examine whether or not neurons in different layers of mouse auditory cortex also show similar spatial preference.

In this study, we investigated the azimuth tuning properties of neurons in mouse AI by applying single unit recordings. We characterized how variations in spike timing of neural responses to seven azimuthal locations are associated with variations in firing rate. We also examined the laminar representation of preferred azimuth direction and azimuth range for each unit isolated along penetrations perpendicular to the cortical surface. Our findings suggest a tendency of columnar organization of optimal azimuthal direction, and significant layer-dependent distribution of azimuth selectivity in mouse AI.

Methods

Animal preparation

A total of 20 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 bw) was given at the beginning of surgery to minimize respiratory congestion. Mice were initially sedated with pentobarbital sodium (60 mg/kg bw) intraperitoneally. A surgical level of anesthesia sufficient to suppress the withdrawal reflex was maintained by continuous subcutaneous infusion of pentobarbital sodium (8 mg/kg bw) throughout the session of an experiment. At the end of the experiment, the animal was given a lethal dose of anesthesia.

Surgery

Once the mouse was anesthetized, a midline incision was made in the scalp. A portion of the scalp and underlying temporal 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 approximately 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. All the experiments were conducted in an anechoic, soundproof, and shielded chamber.

Acoustic stimuli

Stimuli were presented under free-field conditions. The ambient sound pressure level within the chamber was 34 dB (sound pressure level) SPL (A-weighted). The loudspeaker (ES1, Tucker-Davis Technologies, Alachua, Florida, USA; frequency range 2–110 kHz) located 50 cm away was horizontally moveable around the mouse's head. 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° per step).

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, with steps of 1 kHz. Sound intensities were measured at the place of the animal's pinna with a Brüel and Kjaer 4135 microphone (Naerum, Denmark) and measuring amplifier (Brüel & Kjaer Type 2610, Naerum, Denmark) in dB SPL (0 dB SPL re. 20 μ Pa). The characteristic frequency tone that is 20 dB above the minimum threshold (MT) at contralateral 30° of a given neuron was used as stimulus for different azimuths.

Recording sessions

Glass microelectrodes filled with 3 M sodium acetate (impedances approximately 10 M Ω) were carefully positioned orthogonally to the cortical surface by a remote-controlled Pulse Motor Micro-Drive Micromanipulator (SM-21, Narishige, Tokyo, Japan). The electrode was lowered into the cortex to look for single neurons with an accuracy of 1 μ m. Single-unit recordings were performed along the penetration in response to sounds. An indifferent electrode was placed at the nearby temporal muscles. Action potentials of neurons were thresholded and recorded by Tucker-Davis Technologies 3 System. The depth ranges were used as a predominant criterion after verification with latency in several penetrations and were always in accord with established mouse AI laminar boundaries [11]. All recording locations were in AI, as verified through initial multiunit mapping and determined by the layout of the tonotopic gradient.

Data analysis

The characteristic frequency (CF) was defined as the frequency that produced the maximum response at the lowest SPL presented. The MT was defined as the minimum intensity that could elicit the responses. They were determined with the speaker located at contralateral 30°.

Spikes in the time window of 100 ms after the presentation of sound were calculated. The time course from the onset of the stimulus to the peak of the first spike was defined as onset latency, and was averaged over seven trials for each azimuthal location. The azimuth sensitivity of each neuron was characterized based on two measures: best azimuth (BA), preferred azimuth range (PAR). BA was defined as the sound azimuth at which the neuron fired with a maximum of spike count. The PAR was defined as the azimuth range over which azimuth function values exceeded 75% of maximum responses. Multiple comparison test was carried out with MATLAB, and the value α was automatically corrected.

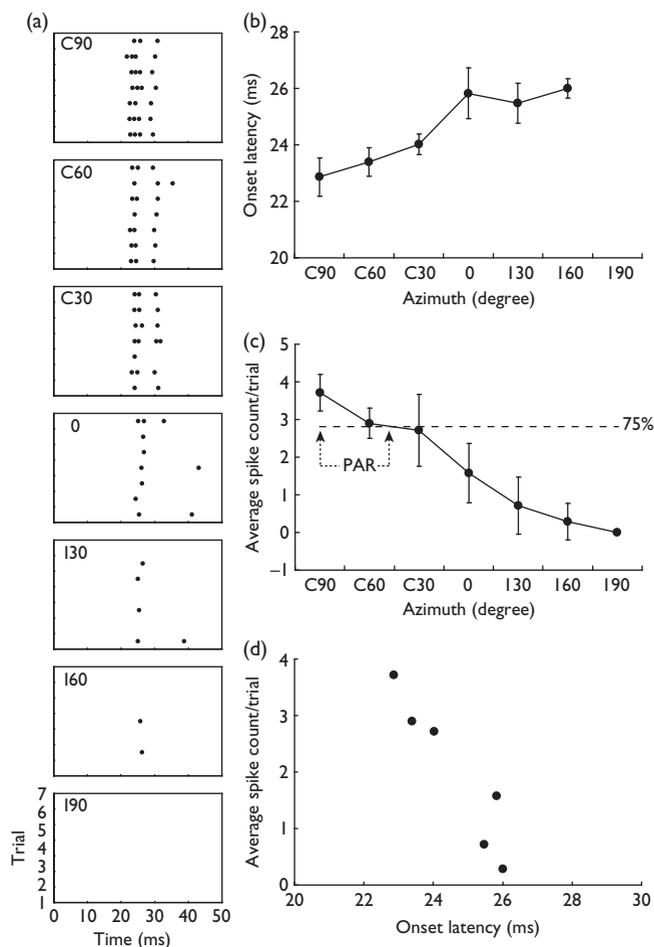
Results

A total of 182 single units from 23 penetrations in the AI of 20 mice were recorded for azimuth-tuning characterization. The CF of the units was predominant from 10 to 24 kHz. Only penetrations with at least 5 units recorded were pooled. Approximately 90% of recorded neurons were sensitive to sound source at contralateral locations.

We examined changes in response onset latency and spike count as a function of azimuthal locations. Figure 1a illustrates the raster plots of the responses of an example unit, which was recorded in layer IV and responded to its CF, 16 kHz, with a brief burst of spikes. We measured the averaged onset latency and spike count for each sound source location over seven trials (Fig. 1b and c). Generally, the onset latency and spike count constantly increased and decreased from C90 to I90, respectively. This type of inverse relationship (Fig. 1d) was observed in approximately 80% of the units recorded. Consistent with the findings by a previous study [20], onset latency in our study typically varied by as much as 4–5 ms across all the azimuthal locations.

We characterized the variation of BA of recorded units along electrode penetrations. The Nissl staining of a representative coronal section of the auditory cortex is shown in Fig. 2a. The left panel of Fig. 2b shows that, from layer II–VI, units in penetration P1 shared the same CF, 17 kHz. The right panel illustrates that, in the same penetration, units shared the same BA, C30, and units in layers III/IV are relatively sharply tuned by azimuths than in other layers. Figure 2c shows the columnar organization of CF of five penetrations (P1–P5). In penetrations P1, P2, and P3, units shared the same BA (Fig. 2d). In our study, 10 of 23 (43.5%) penetrations showed this strict radial organization of BA, and more than 70% of the units

Fig. 1



Azimuth selectivity of example unit recorded in layer IV. (a) Raster plots for responses to different azimuths at 20 dB above the minimum threshold. Each dot represents one spike, and each row represents spikes in one trial. The error bars in (a–c) indicate the standard deviation. (b and c) Spike count and onset latency as a function of sound–source azimuth. Dashed line indicates the 75% of the maximum spike count. Arrows indicate preferred azimuth range. (d) Spike count versus onset latency. Note the inverse relationship between spike count and onset latency.

in all other 13 penetrations shared the same BA (e.g. Fig. 2e).

We also characterized the laminar distribution pattern of PAR. Figure 3a shows the variation of PAR of five example penetrations across different layers. There is some indication that units in layers III/IV were much more narrowly tuned by azimuthal locations than those in layers II, V, and VI. When all the 182 units from 23 penetrations are pooled together, this trend becomes apparent (Fig. 3b). The PARs ranged from 11 to 125°. Statistically, the averaged PARs of units in layers III and IV are significantly narrower than all other layers ($P < 0.05$, multiple comparison test) (Fig. 3c).

Discussion and conclusion

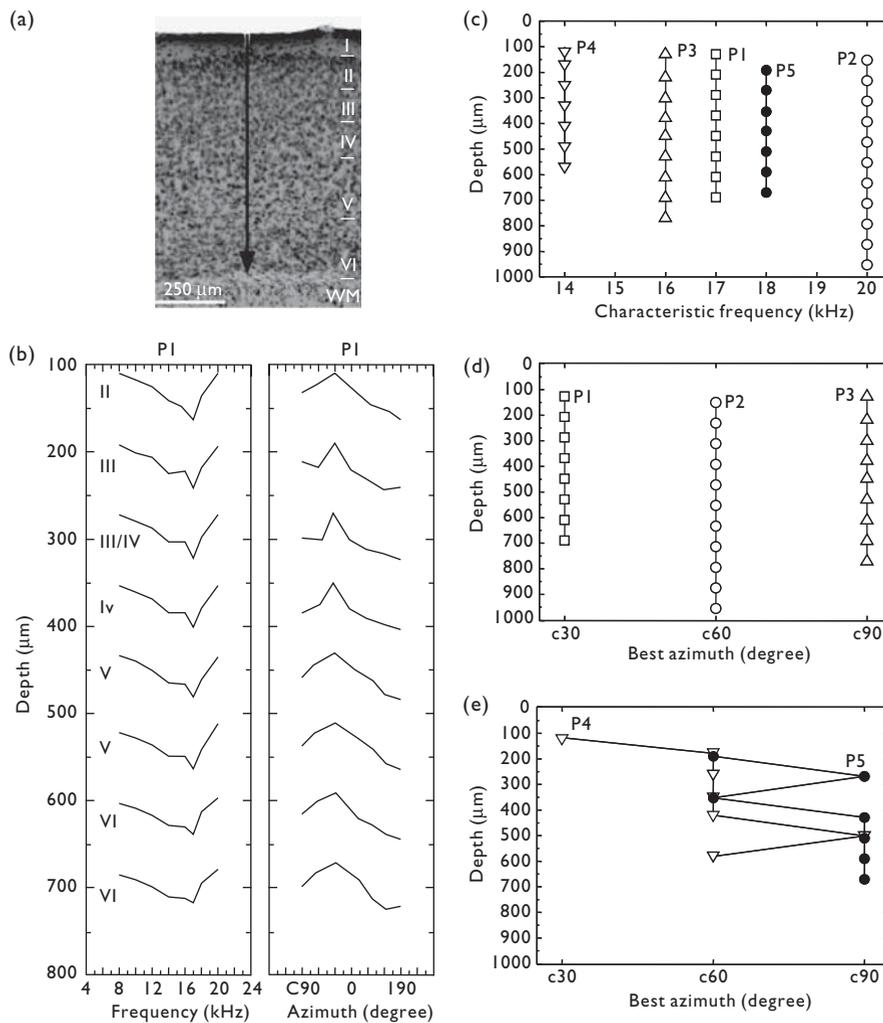
In this study, we investigated the azimuthal tuning properties of mouse AI neurons. We found a significant inverse correlation between onset latency and spike

count, a trend of columnar organization of preferred azimuthal direction, and a significant layer-dependent distribution pattern of selectivity to spatial range.

Studies in cat auditory cortex [8,20] and ferret AI [5] have shown direction-dependent covariation of spike counts and response latency, and indicated that spike timing could be involved as a strategy for spatial coding in the cortex. Our data from mouse AI are broadly consistent with this notion. In our study, approximately 80% of the units recorded demonstrated an inverse correlation between spike count and latency. Thus, our findings about the relationship between spike timing and firing rate showed no significant difference between mouse and other studied species.

It is known that auditory cortical neurons along a radial electrode penetration tend to share similar response properties, including CF [16,17,21], MT [17], intensity

Fig. 2



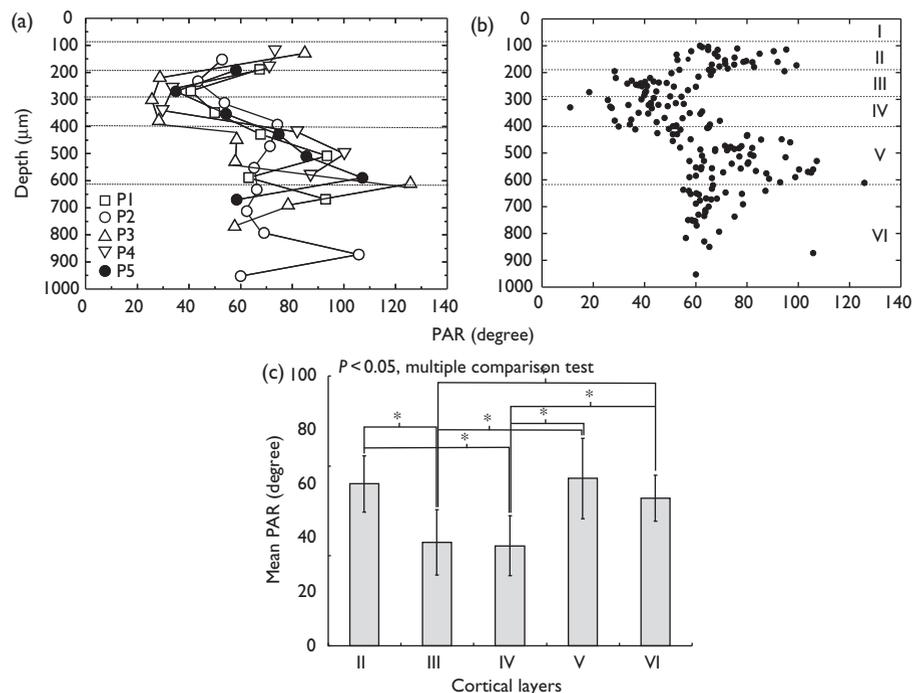
The variation of best azimuth (BA) with penetration depth. (a) Representative mouse auditory cortex coronal section stained with Nissl substance. Black arrow indicates the direction of penetration. Scale bar = 250 μm . (b) Left panel, threshold-frequency tuning curves of the units from penetration P1. The characteristic frequency (CF) is 17 kHz. Right panel, spike count-azimuth tuning curves of the same set of units. The BA is C30. (c) The columnar organization of CF of five example penetrations, P1–P5. (d) Units in three penetrations, P1–P3, share the same BA. (e) The BAs of units in two penetrations, P4–P5, vary with penetration depths.

tuning [22], and frequency sweep tuning [18]. A few studies on either cat or monkey have reported consistent radial organization of response properties related to sound localization [19,22,23]. In our study, we showed a tendency of columnar organization of preferred sound direction in mouse AI. One note of caution is that sampling of the azimuth in our study was fairly coarse. Finer sampling would have been helpful to find out accurate extent of columnar organization of BA.

Layer-dependent variations in first spike latency, frequency tuning bandwidths, spectral and temporal modulation processing have been reported in the auditory cortex of different species [14,15,24]. In terms of spatial selectivity, an earlier study on cat AI [22] has reported

that most neurons encountered in a single normal penetration showed similar PARs. However, we demonstrated a systematic variation of PAR across layers. The notable difference between these two studies may be due to the difference in the sampled cortical depth. We made recordings from layer II through layer VI for most of the penetrations, whereas Clarey *et al.* mainly recorded cells in layer III/IV, which may limit the observation of changes in PAR. The laminar variations in PAR may reflect the reality that layer III/IV receive direct thalamic projections, whereas neurons in the other layers receive substantial horizontal projections. Our findings indicate that the processing of spatial information in AI is predominantly layer dependent with distinct interlaminar diversity.

Fig. 3



The variation of preferred azimuth range (PAR) with penetration depth. (a) The change of PARs of five penetrations with recording layers. (b) Systematic variation of PARs of 182 neurons with layers. (c) The means of PARs of neurons recorded in five layers. Error bars: standard deviations.* The mean PAR of thalamocortical recipient layers, III and IV, are significantly narrower than that of layers II, V, and VI ($P < 0.05$, multiple comparison test).

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