# Topography of acoustic response characteristics in the auditory cortex of the Kunming mouse

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**Abstract** Topography of acoustic response characteristics in the auditory cortex (AC) of the Kunming (KM) mouse has been examined by using microelectrode recording techniques. Based on best-frequency (BF) maps, both the primary auditory field (AI) and the anterior auditory field (AAF) are tonotopically organized with a counter running frequency gradient. Within an isofrequency stripe, the width of the frequency-threshold curves of single neurons increases, and minimum threshold (MT) decreases towards more ventral locations. BFs in AI and AAF range from 4 to 38 kHz. Auditory neurons with BFs above 40 kHz are located at the rostrodorsal part of the AC. The findings suggest that the KM mouse is a good model suitable for auditory research.

**Keywords:** **mouse, auditory cortex, topography of acoustic response characteristics, frequency-threshold curve.**

Frequency spectrum is an essential feature of acoustic signals perceived by animals. To explore the topography of best frequency (BF) and other response characteristics in the auditory cortex (AC) is very important for understanding auditory mechanisms. Extensive studies on frequency representation in AC demonstrate species specialization on frequency ranges in auditory processing. For example, there is a stronger representation of low frequencies in the gerbil[1], middle frequencies in the common marmoset[2], or high frequencies in the echolocating bats[3—7] with distinct BF topography. Our behavioral tests show that acoustic communication has been developed in the KM mouse by calls, the dominant frequency of which is between 10 and 20 kHz with the lowest response threshold of about 5 dB SPL, and perceivable frequency ranged from 1 to 65 kHz. The mouse has a small and smooth cortex which allows a direct analysis of representations for various sound parameters. Stiebler et al.[8] have detailed the differences of left-right frequency representation and tonotopic organization of the AC of anaesthetized house mice (*Mus musculus*) based on multi-unit electrophysiological recordings. However, less is known on topography of acoustic response characteristics of the normal mouse AC.

In the present study, we investigated topography of acoustic response characteristics of neurons in the KM mouse AC, which may contribute to understanding auditory processing mechanisms.

## 1 Materials and methods

(i) Animal preparation. Six male and female KM strain mice (4—5 weeks) weighing 15—18 g with normal hearing, obtained from the Institute of Genetics, Chinese Academy of Sciences, were used. Animals were anaesthetized with sodium pentobarbital (Nembutal, 40 mg/kg b.w.) and the surgery referred to ref. [7]. The flat head of a nail was glued onto the exposed skull of the mouse with acrylic adhesive. Its head was immobilized by fixing the shank of the nail into a metal rod with a set screw. During recording sessions, the mouse was kept awake with the administration of Nembutal (8 mg/kg b.w.) and neuroleptanalgesia (0.08 mg/kg b.w. Fentanyl and 4 mg/kg b.w. Droperidol mixture).

(ii) Recording. Action potentials of single neurons were recorded with 3 mol·$L^{-1}$ KCl-filled glass microelectrodes (impedances about 15 MΩ). An indifferent electrode was placed at the nearby temporal muscles. The electrode was advanced by a remote-controlled Pulse Motor Micro-Drive Micromanipulator (SM-21, Narishige, Japan) with an accuracy of 1 μm. All electrode penetrations were parallel to each other and spaced at ~250 μm. The position of the microelectrode tip was determined with reference to lambda on the skull. The neural activity was amplified (List-electronic L/M-1, Germany), fed to a Dual-beam Memory Oscilloscope (VC-10, Nihon Konden, Japan), a Histogrammer (DAB-1100, Nihon Konden, Japan), and a special computer (DATA 6000 Universal Waveform Analyzer, Data Precision) for acquisition of post-stimulus-time histograms (PSTH) and other data. Experiments were carried out in an electrically shielded, sound-proof and anechoic chamber (temperature 24—26℃).

(iii) Acoustic stimuli. Acoustic stimuli were tones of known frequency that were shaped into tone bursts of 60 ms duration including rise and fall times of 5 ms each, and delivered at a rate of 1/s by a stimulator programmer (HI-MED 100 Series, England). Tone bursts were amplified by a power amplifier, and the resulting outputs led to a broadband loudspeaker (EAS-10TH800B, Matsushita Technic, Japan, frequency range 3—100 kHz). The loudspeaker was placed at a distance of 50 cm to the ear contralateral of the AC from which data were taken. After the experiments were finished, sound intensities of tones within the frequency range presented were determined at the place of the animal's pinna with a calibrated microphone and measuring amplifier (Brüel and Kjaer 4135 and 2610). By this calibration, the response thresholds could be expressed in dB SPL (0 dB SPL re. 20 μPa).

(iv) Data analysis. The best frequency (BF, the sound frequency which elicits a neuron's response at the lowest stimulus intensity), the minimum threshold (MT), frequency tuning curve, and response latency under the intensity of 20 dB above MT of each isolated AC neuron were determined by systematically adjusting the intensity and frequency of the stimulus. The PSTHs illustrate the temporal discharge pattern of each neuron in response to presented stimuli and the total number of impulses. Topography of acoustic response characteristics was studied based on responses of the neurons at different locations of the same animal.

At the end of the experiments, animals were decapitated and the brains were removed. Under a microscope, electrode penetration sites were measured and drawn on graph paper to scale. The border of the AC was determined by connecting mid-points between recording sites with neural responses to tone bursts and the next more peripheral electrode sites without any auditory response. Different fields of the AC were distinguished mainly by their frequency arrangement which could show a continuous frequency shift or no regular tonotopic order. The isofrequency contour lines were drawn in the AI and AAF on a 10-kHz stripe.

## 2 Results and discussion

Data on the acoustic response characteristics of single auditory neurons were limited because previous studies were based on multi-unit electrophysiological recordings from the AC of anaesthetized animals[1,8—10]. The present study was based on response properties of single auditory cortical neurons recorded from awake animals, and the data on frequency selectivity, MT, response latency and firing patterns can be obtained for analyzing the topography of acoustic response characteristics in the AC.

(i) Position of AC and arrangement of auditory fields. Neurons were determined in response to tone bursts with electrode penetrations orthogonal to the cortical surface. The auditory cortex (AC) of the KM mouse was located at the caudal half of the temporal cortex. It locates nearly 1 mm from the occipital end of the cortex, from 1.2 to 3.7 mm rostrally and from 3.0 to 5.0 mm laterally from the lambda (L) (fig. 1). Its area is about 2.0 mm×2.3 mm. One large vein runs from caudal to rostral and branches within the AC, which could provide a rough orientation. Acoustic responses of auditory neurons were recorded from 54 electrode penetrations, as shown in fig. 2, and numbers (in kHz) indicate the BFs of neurons at the appropriate sites. 5 fields of the KM mouse AC (in dashed lines) could be delimited based on BFs arrangements, named primary auditory field (AI), anterior auditory field (AAF), dorsoposterior field (DP), second auditory field (AII) and ultrasonic field (UF).

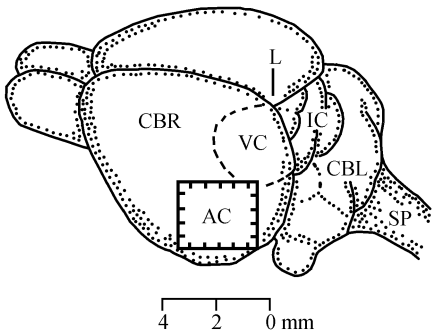


Fig. 1. A dorsolateral view of the exposed brain of the KM mouse. The auditory cortex (AC) is within the rectangle. The lambda (L) represents the reference point for the abscissa and ordinate. The ordinate represents a distance of 3.0—5.0 mm, and the abscissa represents a distance of 1.2—3.7 mm. CBR, cerebrum; CBL, cerebellum; IC, inferior colliculus; SP, spinal cord; VC, visual cortex.

The AI is located in the caudal part of the AC with about 35% of the total AC area. Auditory responses of single neurons within AI were easily elicited and characterized by a phasic pattern of discharges with less than 5 impulses per stimulus, short-latency (10—16 ms), well defined BFs and less

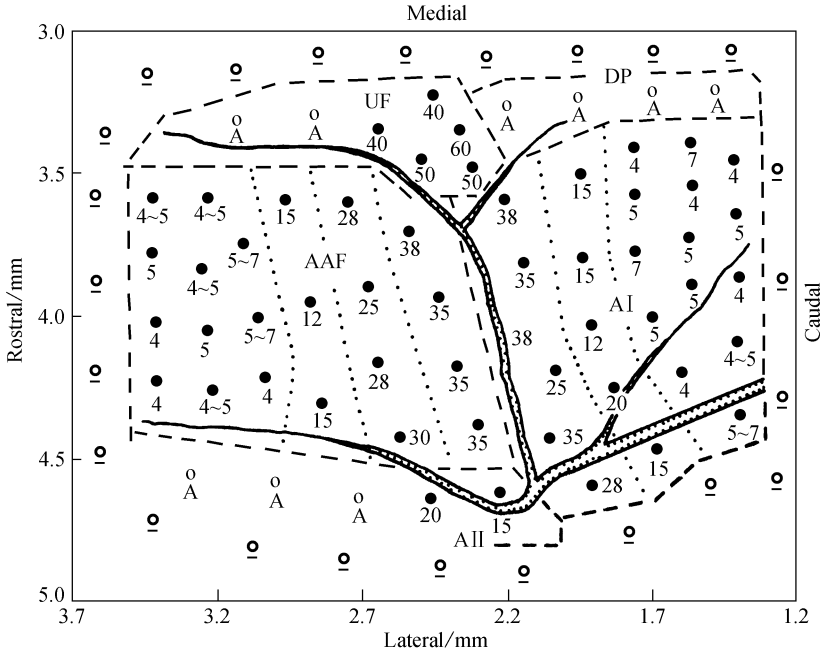


Fig. 2. An enlargement of the rectangular area in fig. 1. ●, Positions of 54 electrode penetrations. Numbers are BFs in kHz measured at the point indicated. "A" means that responses to tone bursts were present but a BF could not be determined. ○, At such locations tone responses were not detected. Heavy dashed lines indicate the boundaries between auditory fields, dotted lines are isofrequency contours. Main blood vessels are stippled. AⅠ, primary auditory field; AⅡ, second auditory field; AAF, anterior auditory field; DP, dorsoposterior field; UF, ultrasonic field.

spontaneous activity. BF, MT and frequency bandwidth varied geometrically. BFs ranged from 4 up to 38 kHz show an orderly tonotopic arrangement with low BFs located caudally and higher BFs rostrally. Isofrequency contours (in dotted lines) sketched in 10-kHz stripe run dorsoventrally. Among them, the low-BFs stripe is disproportionally large, about 40% of the AⅠ area.

AAF, located rostrally to AⅠ, is also characterized by a regular tonotopic frequency representation. BFs in AAF increase from 4 to 38 kHz, inversely to those in AⅠ, i.e., the lowest BFs are located rostrally, the highest caudally. Isofrequency lines of AAF largely run dorsoventrally. Lower BFs below 10 kHz are located rostrally with about 40% of the total AAF area. The higher BFs stripe runs obliquely from dorsorostral to ventrocaudal, and merges with the same stripe in AⅠ.

Neurons with BFs above 40 kHz are located in the dorsorostral region of the AC(UF) as shown in fig. 2. Neither continuous gradient of BFs nor a tonotopic arrangement can be seen in UF. Generally, neurons preferentially respond to complex noise-like sound, and BFs are often difficult to be determined, ranging from 40 to 60 kHz.

DP is located dorsocaudally to AⅠ. Neurons in DP can respond to noise-like sound and have spontaneous pulsed activity. BFs are difficult to be determined. AⅡ is located ventral to AAF. Neurons in AⅡ show rapid habituation in response to sound and a broad frequency range, thus BFs are difficult to be determined as well.

Similar to those found in the house mouse, the AC of the KM mouse could be delimited to 5 subdivisions. However, there are some differences between them: the latter has comparable areas of AⅠ and AAF showing roughly mirror-imaged tonotopic organizations, and UF is small; whereas the AⅠ of the former is larger than the AAF, the dorsal part of AⅠ is situated between the UF and DP, and the area of UF is quite large[8]. Whether these differences in the subdivision fields of AC are species-specific or not needs to be studied further.

(ⅱ) Topography of acousic response characteristics in AⅠ and AAF. Fig. 3 illustrates the

frequency tuning curves of auditory neurons in the low-, mid-, and high- BF isofrequency stripes of AC, their recording positions are indicated in fig. 3(a), respectively. By comparison, the neurons located within the same stripe have similar BFs, but there are significant differences in regard to frequency peaks, MT, response latency and frequency bandwidth.

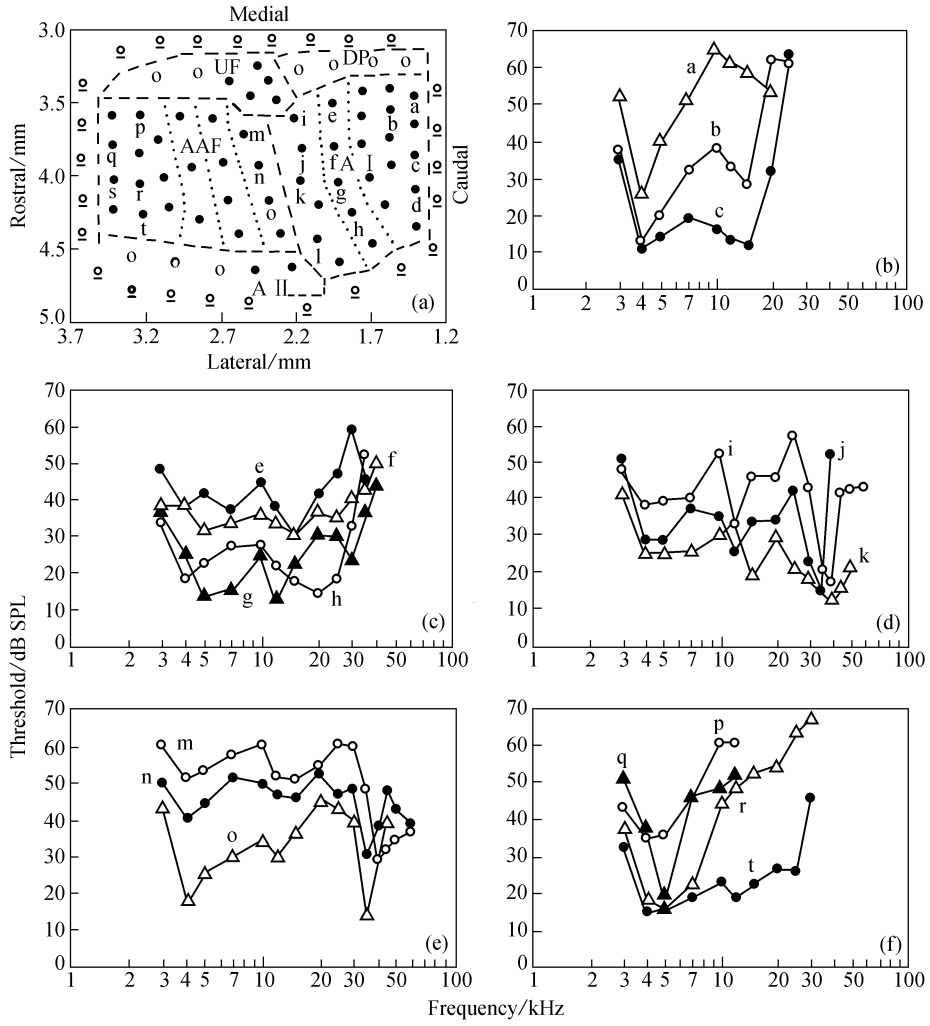


Fig. 3. Frequency-threshold curves of single neurons in A[ and AAF of the KM mouse auditory cortex. (a) A map showing the positions of 20 electrode penetrations, neurons are indicated by letters a—t. ○, At such locations tone responses were not detected. (b)—(f) Frequency-threshold curves of single neurons from different isofrequency stripes, indicated by letters a—t, respectively.

As shown in fig. 3(b), neurons a, b and c located in the low-BF stripe have the same BF at 4 kHz, but MT decreases dorsoventrally from 26 (for neuron a), 14 (neuron b) to about 10 dB SPL (neuron c). The neurons located more ventrally have several sensitive frequency peaks, e.g. neuron c has two peaks at 4 and 15 kHz, and neuron d has three peaks at 4—5, 12 and 20 kHz (not shown in fig. 3(b). Response latency of the neurons increases dorsoventrally from 11.0 (for neuron a), 12.1 (neuron b) to 12.8 ms (neuron c). The measures of sharpness of excitatory tuning curves vary greatly, which is expressed in $Q_{10\ dB}$ value, the quotient of BF and the frequency response bandwidth 10 dB above BF threshold, from 4.4 for neuron a to about 0.3 for both neurons c and d.

The frequency threshold curves of neurons e, f, g and h located in the mid-BFs stripe are shown in fig. 3(c). Evidently, each neuron is broadly frequency-tuned with a frequency range from 4—5 to about

30 kHz. Neuron e has two peaks at 15 kHz (MT=32 dB SPL) and 4 kHz (threshold=40 dB SPL); neuron f has two peaks at 15 kHz (MT=30 dB SPL) and 5 kHz (32 dB SPL); neuron h has two peaks at 20 kHz (MT=14 dB SPL) and 4 kHz (18 dB SPL); neuron g has two peaks located at 12 kHz (MT=12 dB SPL) and 5 kHz (13 dB SPL), and another peak at 30 kHz (22 dB SPL). Response latency of these neurons varies from 13.7 to 15.8 ms dorsoventrally.

Fig. 3(d) shows the frequency tuning curves of neurons i, j and k located in the high-BFs stripe. The BFs are found at 35 and 38 kHz with respective MT of 16.5, 14.6, and 12.4 dB SPL for neurons i, j and k. The next sensitive frequency is at 12 or 15 kHz with the threshold of 6—18 dB above MT. The tuning curves for neurons i and j consist of a high-frequency "valley" with a low-frequency "tail". They are high-frequency bandpass and narrow tuned neuron with the $Q_{10}$ value of about 4. Neuron k is a little broader with $Q_{10}$ of 1.6. These neurons are quite sensitive to sound in the frequency range from 4 to about 40 kHz with a threshold of less than 40 dB SPL. Their response latencies were measured in the range between 13.8 and 14.4 ms.

Fig. 3(e) shows the frequency tuning curves of neurons m, n and o located in the high-BFs part of AAF. BFs are found at 38 and 35 kHz with respective MT of 29.3, 30.7, and 14.6 dB SPL for neuron m, n and o. The next sensitive frequency is at 4 kHz for the ventrally located neuron o with a threshold of 18 dB SPL, whereas the more dorsally located neuron m or n has an increasing threshold. Thus, neuron o is the best sensitive among them in response to sound. Response latency decreases from 16.7 to 11.6 ms in the dorsoventral gradients and relative $Q_{10}$ increases from 2, 4, to about 6 for neurons m, n and o.

Fig. 3(f) shows frequency tuning curves of neurons p, q, r and t located in the low-BFs part of AAF. BFs are found at 4—5 kHz with decreased MT of 34.9, 19.3 16.0 and 15.1 dB SPL for neurons p, q, r and t, respectively, in the dorsoventral gradients seen. The width of the excitatory tuning curve is the smallest for the centrally located neuron q ($Q_{10}$=3.1), a little broad for neuron p or r ($Q_{10}$=1.2 or 1.5), and the broadest for the ventrally located neuron t ($Q_{10}$=0.34). Neuron t has the most sensitive frequency range between 4 and 25 kHz with a threshold difference of about 10 dB. Their response latencies are nearly the same around 14.0 ms, except 16.5 ms for neuron r. In brief, the majority of neurons in the dorsal parts of the AI and AAF have narrow single-peaked tuning curves, and seem to be appropriate to encode perceptually relevant characteristics of frequency resolution and spectral filtering. The ventral neurons have excitatory tuning curves with more than one peak or even two or three separate frequency-response areas with low thresholds. Thus, they do not seem to have such properties. Response latencies of neurons in the AI gradually increase from caudal (e.g. about 11 ms) towards more rostral locations (about 14 ms); that is, cortical neurons with lowere BF usually have short response latency.

There are topographical differences in acoustic response characteristics for neurons of the AI and AAF. In the low isofrequency stripes, neurons tend to have decreasing response thresholds and increasing width of tuning curves in dorsoventral gradients seen. In the middle and high isofrequency stripes, neurons located dorsally have higher response thresholds than those ventrally, broader frequency tuning curves and two or three sensitive frequency peaks. These topographies of acoustic response characteristics on isofrequency stripes of the AI and AAF, together with the tonotopy itself, certainly express much of the sound processing potential of the AI and AAF as a function of the loci. The finding suggests that the KM mouse is a good model for auditory research.

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