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Topography of acoustic response characteristics in the midbrain inferior colliculus of Kunming mouse

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Abstract **Topography of acoustic response characteristics of the midbrain inferior colliculus (IC) of the Kunming mouse was studied by using extracellular recording techniques. The characteristic frequency (CF) range represented in the different divisions of the IC differed markedly: 4—15 kHz in the dorsal cortex (DC), 10—70 kHz in the central nucleus (CN), and 4—35 kHz in the external cortex (EC). The CF in the CN increased from dorsal and lateral to ventral and medial, higher CFs represented at its ventromedial part and lower CFs at its dorsal part. The isofrequency contours of CFs were incurvate. Minimum thresholds (MT) of the auditory neurons in DC and the central part of CN were lower (about 10 dB SPL), but considerably higher in the dorsal and ventral region of EC. Results suggest that each of the divisions in the mouse IC may have different auditory functions.**

Keywords: mouse, inferior colliculus, auditory neuron, characteristic frequency, minimum threshold.

The mammalian auditory system from the cochlea up to auditory cortex possesses the tonotopy, which is of significance for frequency discrimination and recognition. It is an important and open problem how the auditory pathways extract, process and integrate complex frequency information derived from acoustic signals. The inferior colliculus (IC), a center for processing monaural and binaural information, has direct ascending connections from several brainstem nuclei, and is modulated by the auditory cortex via descending connections to the medial geniculate body and IC. It has been demonstrated that the IC plays an important role in the aspects of frequency tuning, a sound source localization and stimulus-duration selectivity[1—8]. It is still unclear how frequency representations are organized in the mouse IC. Previous studies have indicated that the IC in the cat receives major projections from different brainstem nuclei, which dispersed to different locations on the isofrequency planes; the IC represents frequency features through the afferent projections and also may have new functions resulted from the interaction between the separate projections. Stiebler et al. studied tonotopic organization, frequency representation, and tone-threshold distribution in the IC of anesthesized house mice (*Mus musculus*) by using electrophysiological mapping techniques[9]. However, the topography of acoustic response characteristics in the midbrain IC of normal mouse and its functions are less known. Behavioral experiments have showed that Kunming mice produce calls with the dominant frequency between 10 and 20 kHz and respond to sounds of a broad frequency range (1 to 65 kHz) with the minimum threshold of about 5 dB SPL[10]. The present report is to study the topography of acoustic response characteristics in the IC of the mouse and possible functions for different divisions.

1 Materials and methods

(i) Animal preparation and surgery. Three outbred male albino mice (*Mus musculus* Km) (4—6 weeks old, weighing 20—25 g) with good hearing were used and obtained from the Experimental Animal Center of the Institute of Genetics the Chinese Academy of Sciences. The mouse was anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg b. w.) with surgery referred to those in refs. [10,11]. During recording, the mouse was lightly anesthetized with administration of Nembutal (8 mg/kg b. w.) and neuroleptanalgesia. The skull and dura over the left midbrain IC were removed and then the left IC was exposed.

(ii) Recording. A 3-mol/L KCl-filled glass microelectrode (impedance about 10—15 MΩ) was inserted into the IC in an angle of 70° toward the horizontal plane for recording responses from auditory neurons. The first penetration was selected at the center of the IC, apart from the λ point about 800 μm. The frontal section through the center was called the medial plane (m). Other two frontal sections were called the rostral (r) and caudal (c) plane, respectively, each 500 μm rostral and caudal from the m plane. On each plane, a recording microelectrode was parallel penetrated at the interval of 200—500 μm, the first recording position was at about 50 μm beneath the surface layer of the IC, and acoustic responses were measured every 200 μm in depth. A reference electrode was placed at nearby temporal muscles. The electrode was advanced by Micro-Drive micromanipulator (SM-21, Narishige, Japan) with an accuracy of 1 μm. Neuronal activity was amplified by an amplifier (List-electronic L/M-1, Germany) and fed to an oscilloscope (VC-10, Nihon Kohden, Japan) and a special computer (DATA 6000 Universal Waveform Analyzer, USA) for recording and late analysis. Experiments were conducted in an electrically shielded, soundproof and anechoic chamber (temperature 24—26℃).

(iii) Acoustic stimuli. Tone burst of 60 ms duration including rise and fall times of 5 ms each at a rate of 1/s was generated by a Stimulator Programmer (Hi-Med 100,

England) and power amplified to a speaker (EAS-10TH800B, Matsushita Technic, Japan, frequency range 3—100 kHz) located on the right side of the mouse, 50 cm apart from its right ear. Sound intensities of tones were determined at the place of the mouse's pinna with a microphone (Brüel & Kjaer 4135, Denmark) and measuring amplifier (B&K 2610), expressed in dB SPL (re. 20 μPa r.m.s.).

(iv) Data analysis. Frequency tuning curve of an auditory neuron responded to contralateral stimuli, characteristic frequency (CF), minimum threshold (MT) and response latency under the level of 20 dB above MT and at CF of each neuron were measured by systematically adjusting the intensity and frequency of the stimulus. The acoustic response characteristics of auditory neurons at different positions on three frontal planes (r, m and c) from the same animal were analyzed.

2 Results

31 penetrations were conducted in IC of 3 mice. 256 sites were tested for acoustic responses and 152 complete multi-unit frequency-tuning curves were obtained for the analysis of CF, MT, Q_{10} and latency.

(i) Topography of CF in the IC. Fig. 1 shows the mappings of auditory neurons' CF in the IC of mouse. On the r, m and c frontal planes, there were obvious changes in CF along the penetration of microelectrode from dorsal to ventral. From the dorsal cortex (DC) into the central nucleus (CN), CF rose stably with increasing depth: from 4, 8 or 15 kHz up to 18, 25 or 30 kHz on the r and m planes; to 50, 60 or even 70 kHz within the medial part of CN (M). But from CN into the external cortex (EC), CF decreased suddenly to 10 or 8 kHz, even less. Responses to tone bursts were absent in one track situated outside of the m plane through the EC. On the c plane, similar changes of the CF with the increasing depth were seen, but not continued in the EC. From the lateral to the medial side of the IC, CF increased on the whole. Penetrations into the periaquaeductal gray and interstitial nucleus of the IC did not reveal any responses to acoustic stimuli.

The CFs of auditory IC neurons in the mouse range from 4 to 70 kHz, thereinto from 4 to 15 kHz for the DC, from 10 to 70 kHz for the CN including the M part, and from 4 to 35 kHz for the EC. The lowest CF (4 kHz) is mainly found beneath the surface layer of IC from 50 to 200 μm, and scattered over 2000 μm in the EC as well. The highest CF (60—70 kHz) is mainly distributed in the M area.

(ii) Isofrequency contours in the IC. The isofrequency contours, which connect points of the same CFs in different penetrations within the same frontal plane in the IC, demonstrated that the tonotopic organization of the IC in the mouse showed a kind of laminar structure pattern (Fig. 2). Within the m plane, as shown in Fig. 2(b), the 8-kHz isofrequency line in the DC was considerably flat, but that in the CN showed the slopes varying with increasing CF from 18- to 60-kHz. The isofrequency lines of different CFs in the r plane were steeper than those in the m plane, but those in the c plane were not steep as others. The CFs scattered in the EC showed irregular changes, and it was hard to draw isofrequency contours in the EC.

2-D isofrequency contours on different frontal planes allowed to the reconstruction of 3-D isofrequency laminae in the IC with an onion-like shape, similar to those in the house mouse[9]: the isofrequency planes of different CFs were organized from outside to inside with increasing frequency. The slopes of different isofrequency laminae increased from dorsal to ventral and from caudal to rostral. Among them, the laminae of 20—40 kHz and 12—18 kHz occupied a larger space in the IC, which corresponded with the amounts of auditory neurons of different CFs: 40.2% in the CF range between 20 and 40 kHz,

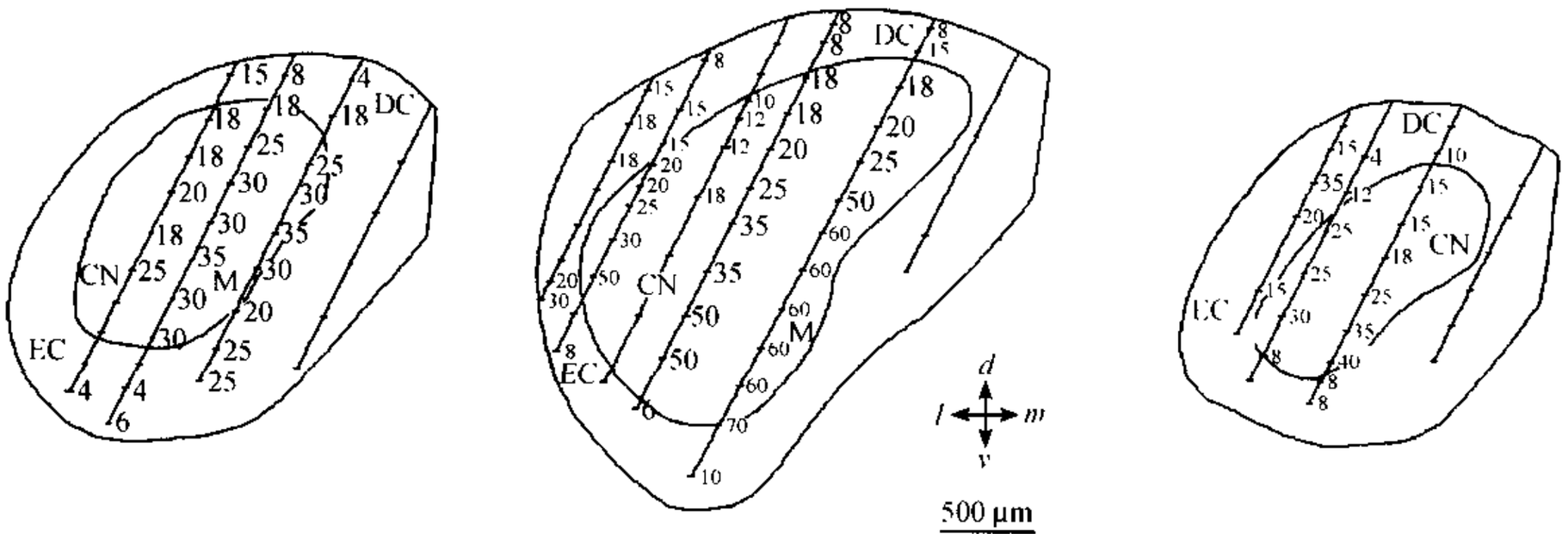
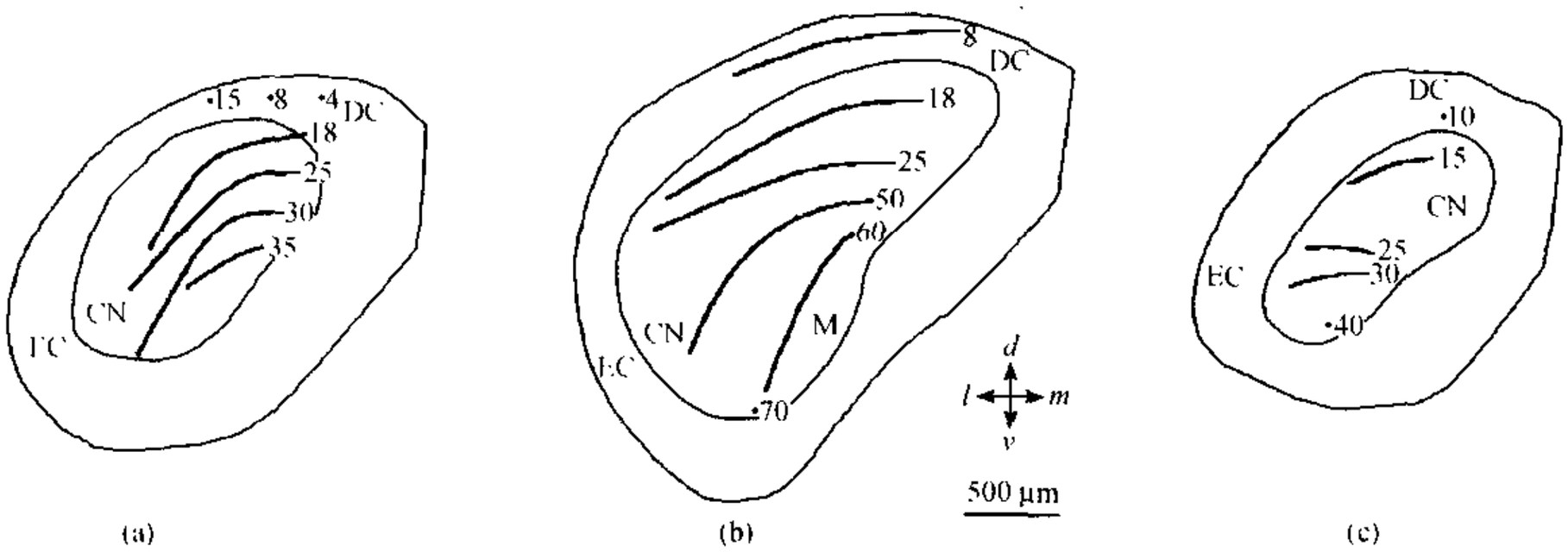


Fig. 1. Characteristic frequency mappings in the inferior colliculus of the mouse. (a) r section; (b) m section; (c) c section. DC, dorsal cortex; CN, central nucleus; M, medial part of CN; EC, external cortex; *d*, dorsal; *v*, ventral; *l*, lateral; *m*, medial, the same below. Unit: kHz.



(a) (b) (c)

Fig. 2. Isofrequency contours of CF for auditory neurons in the inferior colliculus of the mouse. (a) r section; (b) m section; (c) c section. Unit: kHz.

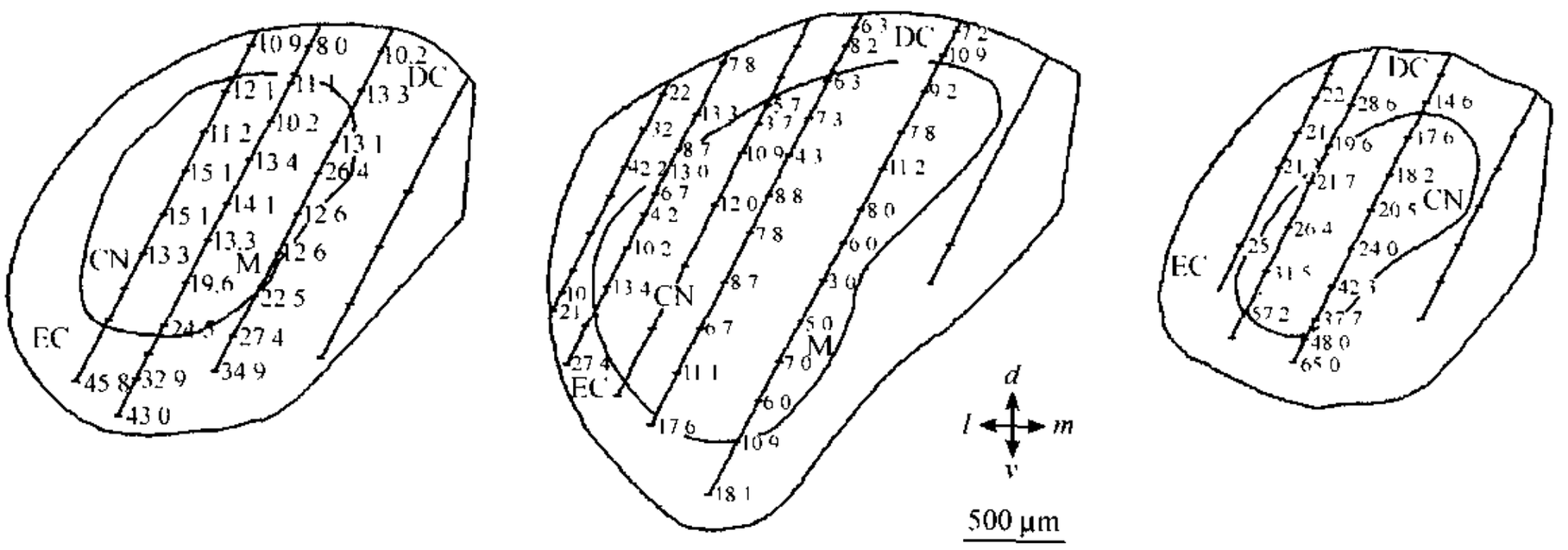
29.6% between 12—18 kHz, 17.1% between 8—10 kHz, 9.2% above 45 kHz, and 3.9% below 5 kHz (a total of *neurons*, $N = 152$).

(iii) Minimum threshold distribution in the IC. Fig. 3 illustrates the distribution of MTs on the different frontal planes. The MTs of auditory neurons in the DC and CN on the m plane were lower (mean $\pm$ SD, the same below), about 8.10 ± 1.55 ($n = 5$) and 8.20 ± 2.70 ($n = 29$) dB SPL, respectively, but higher of 22.6 ± 9.47 ($n = 9$) dB SPL in the EC (Table 1). The cases on the r and c planes were similar to those on the m plane. The MT of auditory neurons located at the ventral part on the c plane was the *highest about 65 dB SPL. Statistical analysis shows that* there are significant differences in the MT means between EC and CN, and between EC and DC ($t = 6.79$, and 4.87, respectively, $P < 0.001$). On the same isofrequency laminae, auditory neurons of lower MT and higher sensitivity were located in the center of the laminae.

Table 1 Minimum thresholds (dB SPL) of auditory neurons on the frontal planes of the IC in the mouse (total number of neurons, $N = 91$)

Frontal section	DC		CN		EC	
	MT	*n*	MT	*n*	MT	*n*
r	10.6 ± 1.89	4	14.2 ± 4.52	16	31.3 ± 8.13	6
m	8.10 ± 1.55	5	8.20 ± 2.70	29	22.6 ± 9.47	9
c	12.2 ± 2.40	2	22.1 ± 7.77	11	36.4 ± 16.7	9

(iv) Frequency-tuning sharpness and response latency in the IC. The frequency-tuning sharpness of auditory neurons in the IC, expressed in $Q_{10\,dB}$ value (the quotient obtained by dividing CF by the frequency band width of the neuron's tuning curve at 10 dB above MT), ranged from 0.48 to 5.83. The Q_{10} values for the neurons with high CF or low MT were relatively high (Fig. 4). Table 2 shows the variations of average Q_{10} values for auditory neurons on different frontal planes in the IC. Q_{10} values were great for the neurons in the core of CN, espe-



(a) (b) (c)

Fig. 3. Distribution of minimum thresholds for auditory neurons in the inferior colliculus of the mouse. (a) r section; (b) m section; (c) c section. Unit: dB SPL.

cially in the M area. If the neurons had the same CF, Q_{10} values denoted the frequency selectivity. Auditory neurons of high Q_{10} were concentrated in the center of isofrequency laminae, which overlapped the lowest MT area. It indicates that the auditory neurons located in the center of the IC have both high sensitivity and frequency selectivity, which are very suitable for frequency discrimination and weak sound detection.

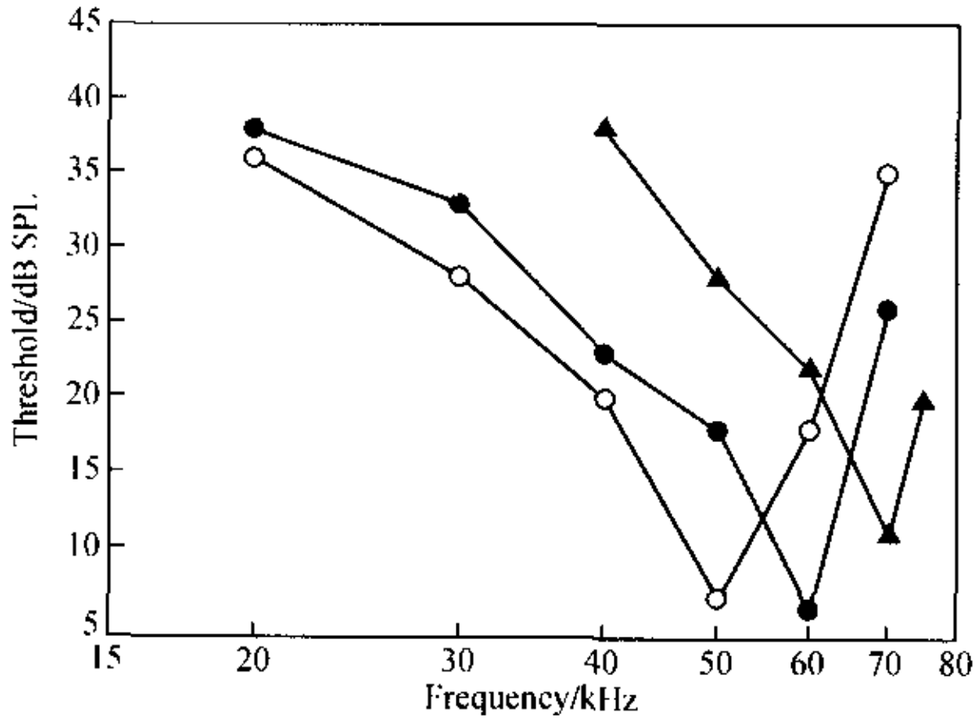


Fig. 4. Frequency tuning of 3 auditory neurons in the M area of the m plane of CN in the IC of the mouse. CF (kHz), MT (dB SPL) and $Q_{10\,dB}$: ○, 50, 6.7, 3.12; ●, 60, 6.0, 4.61; ▲, 70, 11, 4.67.

Table 2 Variations of frequency-tuning sharpness (Q_{10}) of auditory neurons on the different frontal IC sections in the mouse (total of neurons, $N = 91$)

Frontal section	DC		CN		EC	
	Q_{10}	n	Q_{10}	n	Q_{10}	n
r	1.19±0.25	4	2.27±0.78	16	1.98±0.52	6
m	1.23±0.48	5	2.30±1.14	29	2.07±0.58	9
c	0.72±0.14	2	1.39±0.34	11	1.44±0.50	9

The auditory neurons of the longest latency of about 15.0 ms, measured at CF and the level of 20 dB above the threshold, were found in the surface layer of the DC, and those of the shortest latency of 5.3 ms located at the bottom of the IC. The response latency decreased from dorsal to ventral with a rate of about 0.3 ms/100 μm. It seems that no regular changes in response latency of the auditory neurons were found on different frontal planes of the IC.

3 Discussion

The present study revealed the characteristic frequency mappings and the distribution of response thresholds in the auditory midbrain inferior colliculus of the mouse and extended the knowledge of functional organization of mammalian IC by using electrophysiological techniques. Compared with the IC of house mouse[9], auditory neurons of the IC of Kunming mouse have lower spontaneous activities (firing rate < 5 spikes/s). The high signal noise ratio is favorable to transmission and processing of auditory information. The frequency representation ranges in different divisions of IC in the mouse are markedly different, as in other experimental mammals. The CF ranges represented in the DC and CN of the mouse's IC are basically continued, and constitute its total hearing range (4—70 kHz), which is represented by a single tonotopically set of isofrequency laminae bending from dorsal to ventral. The CF range represented in the EC is narrow (4—35 kHz) and scattered. It implies that different divisions of the IC share out auditory functions. The auditory neurons in the DC with high sensitivity and long latency may mainly process low frequency sound. The CN occupies a large part of the IC, in which the auditory neurons with highest sensitivity and short latency form the core of the IC for the reception and processing of acoustic information. The EC with a narrow CF range and high threshold is possibly relative to the integration of auditory and somatosensory responses[11,12]. For example, licking responses in mouse mother evoked by wriggling sound of mouse pups are very frequently enhanced by somatosensory stimulation of moving pups[9].

As mentioned above, the finding that auditory neurons in the CN of the midbrain IC in the mouse possess evident acoustic characteristics (i.e. lower threshold, high CF and short latency), is quite similar to those in the horseshoe bat (*Rhinolophus ferrumequinum*)[13]. Flying horseshoe bats determine relative speed and spatial location of the prey by the Doppler shift of the constant frequency component contained in the echolocating signals. Most neurons responsible for this kind of important behavior are tuned to the constant frequency and concentrated in the ventromedial region of the IC, anatomically similar to the CN of IC in the mouse.

Furthermore, unlike mammals including cats, the auditory cortex in the mouse includes a special area called the ultrasonic field (UF)[10], in which auditory neurons have the CF > 40 kHz. Experiments on acoustic behavior in the mouse showed that mouse pups calls contain high frequency components (> 40 kHz). It can be speculated that the main features of calls may be extracted and processed by the neurons in the CN of IC in the mouse, and then transferred to the auditory cortex for recognition. It is not clear how the IC neurons of CF > 40 kHz are projected into the UF of the auditory cortex through the medial geniculate body, and affect acoustic behavior in the mouse.

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Fine mapping of the *Ht2* (*Helminthosporium turcicum* resistance 2) gene in maize

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Abstract **Fine mapping of *Helminthosporium turcicum* resistance gene *Ht2* is extremely valuable for map-based cloning of the *Ht2* gene, gaining a better knowledge of the distribution of resistance genes in maize genome and marker-assisted selection in maize breeding. An F_2 mapping population was developed from a cross between a resistant inbred line 77*Ht2* and a susceptible inbred line Huobai. With the aid of RFLP marker analyses, the *Ht2* gene was mapped between the RFLP markers UMC89 and BNL2.369 on chromosome 8, with a genetic distance of 0.9 cM to BNL2.369. There was a linkage between SSR markers UMC1202, BNLG1152, UMC1149 and the *Ht2* gene by SSR assay. Among the SSR markers, the genetic distance between UMC1149 and the *Ht2* gene was 7.2 cM. By bulked segregant analysis 7 RAPD-amplified products which were probably linked to the *Ht2* gene were selected after screening 450 RAPD primers and converted the single-copy ones into SCAR markers. Linkage analysis showed that the genetic distance between the SCAR marker SD-06_{633} and the *Ht2* gene was 0.4 cM. From these results, a part of linkage map around the *Ht2* gene was constructed.**

Keywords: **maize, *Helminthosporium turcicum* resistance gene *Ht2*, fine mapping, genetic map, molecular markers.**

Northern corn leaf blight (NCLB), caused by *Helminthosporium turcicum* (Pass.), is a major disease of maize. Several genes, *Ht1*, *Ht2*, *Ht3* and *HtN* have been reported to be responsible for resistance to specific races of the pathogen. At present, hybrid corn in China is usually resistant to race 1, but highly susceptible to race 2. With global temperature increasing, the epidemic of race 2 would take place easily. It is important to get multigenic resistance in one genotype for breeding maize cultivars with high resistance to NCLB. The *Ht1* and *Ht2*, *HtN* genes were located on different chromosomes of maize. By routine breeding, pyramiding them in one elite genotype needs a long period and shows low efficiency, meanwhile, the genes of undesirable traits would unavoidably be carried into the genotype. Moreover, when the plants carrying the *Ht2* gene and those carrying both *Ht1* and *Ht2* genes are inoculated with race 2, they would