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# The Effect of Monaural Middle Ear Destruction on Postnatal Development of Mouse Inferior Colliculus

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## Abstract

**This study examined the effect of monaural middle ear destruction on postnatal development of inferior collicular (IC) neurons of the laboratory mouse, *Mus musculus*. Monaural middle ear destruction was performed on juvenile mice and the density, number and size of IC neurons were determined at different post-operative ages. For electrophysiological study, collicular auditory response properties were always examined four weeks after operation. Monaural middle ear destruction produced larger neurons in the ipsilateral IC (relative to the operated ear) and smaller neurons in the contralateral IC of experimental mice in comparison with IC neurons of control mice. IC neurons of control mice typically had lower minimum thresholds and greater $Q_{10}$ values than IC neurons of experimental mice. In experimental mice, neurons in the contralateral IC typically had longer latencies and higher minimum thresholds than neurons in the ipsilateral IC. Clear tonotopic organization was only observed for IC neurons of control mice. Possible mechanisms for these different observations are discussed.**

**Key Words: frequency tuning curve, inferior colliculus, mice, middle ear destruction, tonotopic organization, soma size**

## Introduction

In the ascending auditory pathway, the inferior colliculus (IC) receives excitatory and inhibitory inputs from all lower auditory nuclei (1,2,21,22,25-27,29). These excitatory and inhibitory inputs contribute importantly to auditory temporal processing in this nucleus (7). Previous studies have demonstrated that abnormal auditory stimulation during early postnatal development can be manifested through anatomical and physiological changes that occur in the IC. For example, electrophysiological studies have shown that monaural plugging permanently changes the auditory spatial sensitivity of neurons in the IC of bats (15) and produces substantial loss of binaural interaction in the IC of rats (8,9,32). Unilateral cochlear removal produces an increased proportion of excited contralateral IC recording loci, lower minimum thresholds (MTs), wider dynamic ranges, higher peak discharge rates, and more tonic responders to sound stimulation of the intact, ipsilateral ear when compared with responses of IC neurons of the control animals with both cochleas intact (3,16,17,19). Conversely, early stimulation with a specific sound frequency in mice and rats results in a large number of IC neurons that are tuned to the early experienced sound frequency (10,11,23,24,28).

To further determine the effect of abnormal early

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auditory experience on postnatal development of the IC, we studied postnatal development of IC neurons in mice under monaural middle ear destruction using histology and electrophysiology. We report here that monaural middle ear destruction produced larger neurons in the ipsilateral IC (relative to the operated ear) and smaller neurons in the contralateral IC of experimental mice. Electrophysiologically, neurons in the contralateral IC typically had longer latencies and higher MTs than neurons in the ipsilateral IC. Clear tonotopic organization was not observed for either IC neurons of experimental mice. Preliminary reports have been presented earlier (39,40).

## Materials and Methods

A total of 52 juvenile laboratory mice, *Mus musculus*, was used for this study. For experimental mice, monaural middle ear destruction was performed at the day of natural opening of the ear canal (usually 13 days after birth, DAB). A pair of fine forceps was inserted through the ear canal under the light microscope to remove the tympanic membrane and the ossicular chain of each Nembutal anesthetized (50 mg/kg b.w.) juvenile mouse. The operated mice were then observed under a heat lamp until they completely recovered from anesthesia. This monaural middle ear destruction represented an acoustic manipulation or conductive modification which severely reduced the sound intensity reaching the inner ear (33,35,36).

For histological study, 12 juvenile mice with (experimental) or without (control) monaural middle ear destruction were raised for different periods of time after natural opening of the ear (15, 30 or 45 days or at the age of 14-28, 14-43 or 14-58 DAB). At the end of each experimental period, each mouse was sacrificed. Its brain was removed from the skull, fixed in 10% formalin solution, and processed for cresyl violet staining. Briefly, each brain was washed with distilled water and dehydrated in 50%, 75%, 80%, 95% and 100% alcohol before being cleared with xylene, infiltrated and embedded with paraffin. Each brain was coronally sectioned at a thickness of 10 μm before being mounted onto gelatin coated slides. All slices were then dehydrated in alcohol, stained with cresyl violet, differentiated, cleared in xylene, and covered with a cover slip using permount. A total of 288 sections was cut from each IC and they were mounted on 24 microscopic slides (i.e. 12 sections/slide).

Procedures for determination of neuronal density, number and soma size of IC neurons in each mouse were similar to previous studies (4,37). Briefly, each histological brain section was divided into 4 quarters using an eyepiece micrometer and a light microscope at the magnification of 400x (Nikon) and then the number of IC neurons within a randomly selected quarter was counted. The IC neurons counted all had cytoplasmic staining for Nissl substance, with a distinct nucleus and nucleolus. The total number of neurons in each brain section was then estimated from the counted number multiplied by four. Counting of IC neurons was performed for all 288 brain sections from each IC and was restricted to the same region in both ICs and approximately the same region across all mice. The average number of IC neurons per slide (i.e. per 12 brain sections) was obtained for each experimental condition from both groups of mice. The difference in the average number from different experimental conditions was statistically compared using one-way ANOVA or t test.

Neuronal density (number of neurons per $mm^2$) was obtained by dividing the counted number of IC neurons by the cross-sectional area of the cytological region that containing the counted IC neurons. Cross sectional soma size was determined by outlining the stained portion of neurons with a recognizable nucleolus using a Javelin 12-inch Video interactive image-analysis system (Javelin Electronics, Torrence, CA), an eyepiece micrometer and a light microscope (Nikon). The long and short axes of 10 randomly selected IC neurons in each slide were measured. Soma size was approximated as half of the sum of the long and short axes of each neuron. Soma size was measured in 480 IC neurons (240 neurons in each IC) for each mouse and a total of 5760 IC neurons was measured from all 31 mice. Soma sizes for neurons from control and experimental mice were statistically compared using one-way ANOVA or t test.

Electrophysiological study were conducted at four weeks after monaural ear destruction in experimental mice and at approximate the same age in control mice (i.e. 41-45 DAB). Procedure for surgery were the same as in a previous study (5). Briefly, each of 44 mice was anesthetized with Nembutal (70 mg/kg b.w.) plus the neuroleptanalgesic Innovar-Vet (0.08 mg/kg b. w. of fentanyl, 4 mg/kg b. w. of droperidol). The flat head of a 1.8 cm nail was then attached to the exposed skull with acrylic glue and dental cement. A hole was drilled through the skull overlying the IC. A local anesthetic (lidocaine) was applied to the open wound area. The mouse was strapped to an aluminum

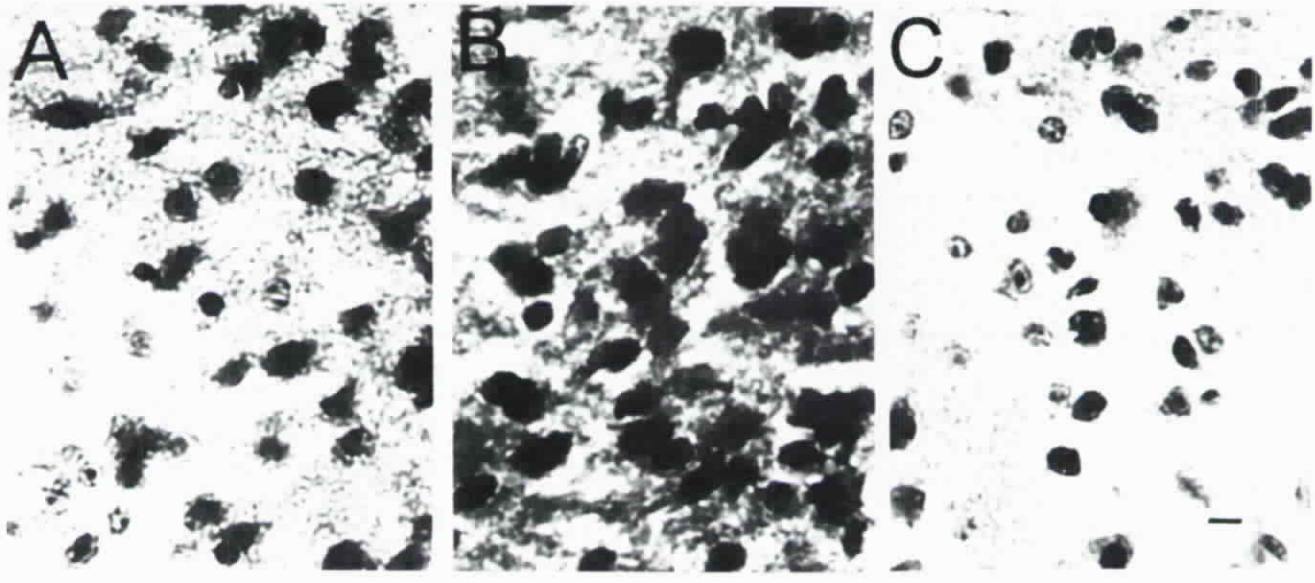


Fig. 1. Photomicrographs of cresyl violet staining showing inferior collicular (IC) neurons from control mice (A) and experimental mice (B,C) examined at age of 58 days after birth. In comparison with the neurons of control mice (A), neurons in the ipsilateral IC of experimental mice had the largest soma size (B) and neurons in the contralateral IC had the smallest soma size (C). Scale bar:6 μm.

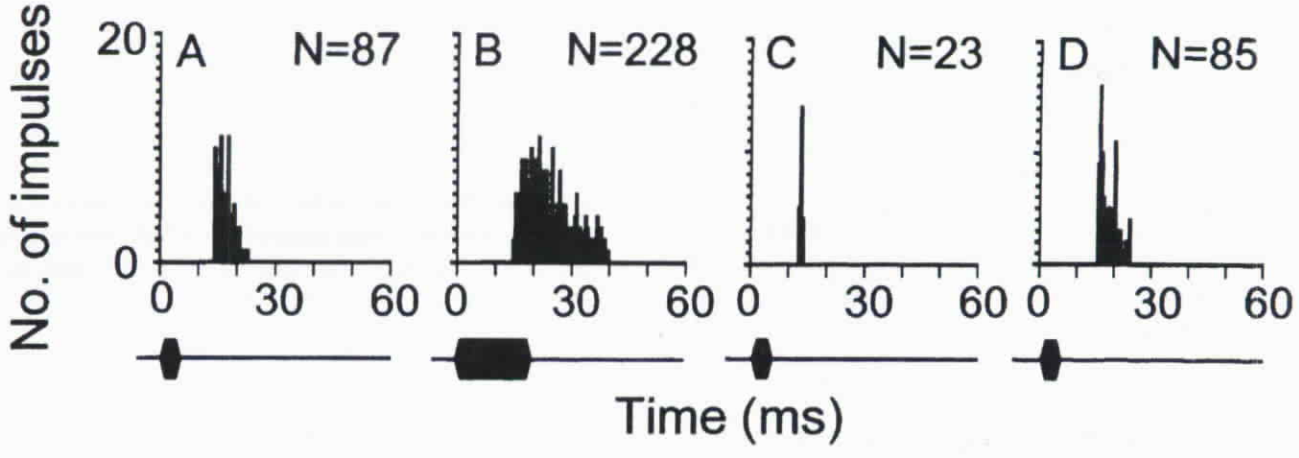


Fig. 2. Peri-stimulus-time (PST) histograms showing the discharge patterns of 3 representative IC neurons in response to best frequency (BF) sounds delivered at 10 dB above the minimum threshold (MT). The tonic responder discharged impulses throughout the entire duration of the acoustic stimulus (A,B). The phasic responder (C) discharged 1-2 impulses and the phasic burster (D) discharged 3-5 impulses to presented acoustic stimuli. BF sounds were 4 ms in A,C and D but were 20 ms in B. N: number of impulses discharged to 16 stimuli. Bin width: 500 μs, sampling period: 300 ms. For convenience, only a part of PST histogram (up to 60 ms ) is shown. The BF (kHz), MT (dB SPL), recording depth (μm) and latency (ms) of these neurons were 7.1, 35,760,14 (A,B);13.4, 45,1150,12 (C); 19.1, 65,1490,16 (D).

holder and placed inside a sound-proof chamber (temperature 28°-30°C) whose ceiling and inside walls were covered with 3-inch convoluted polyurethane foam to reduce echoes. After orienting the mouse with its eye-snout line pointed to 0° in azimuth and 0° in elevation of the frontal auditory space, its head was immobilized by fixing the shank of the nail into a metal rod with a set screw (34). Glass electrodes of 3M KCl (impedance: 2-5 MΩ) were inserted into the IC to record sound-evoked neural responses. Recording depth was read from the scale of a microdrive (David-Kopf). An indifferent electrode was placed at the nearby temporal muscles. Each mouse was used for 1-6 recording sessions on separate days and each recording session typically lasted for 4-6 hours.

To generate acoustic stimuli, continuous sine waves from an oscillator (KH model 1200) were formed into tone pulses (4 ms, 0.5 ms rise-decay times, at 2

**Table 1. Density, Number and Size of Inferior Collicular Neurons of Experimental and Control Mice Determined at Different Postnatal Periods with or without Monaural Middle Ear Destruction**

| | | | Postoperative periods | | | |
|---|---|---|---|---|---|---|
| | | | 15-day | 30-day | 45-day | ANOVA *p* |
| **Density no/mm$^2$** | | | | | | |
| | control | | 4040 | 4104 | 4145 | |
| | expt | (ipsi) | 4023 | 3991 | 4043 | |
| | | (contra) | 3154 | 2840 | 1806 | |
| **Number m±sd*** | | | | | | |
| | control | | 111±2.8 | 112±3.4 | 113±4.6 | <0.0001 |
| | expt | (ipsi) | 110±3.7 | 109±3.7 | 110±2.4 | <0.0001 |
| | | (contra) | 83.8±4.8 | 77.5±2.8 | 49.3±4.3 | <0.0001 |
| **ANOVA** | | *p* | <0.0001 | <0.0001 | <0.0001 | |
| **Size (μm) m±sd**** | | | | | | |
| | control | | 5.45±1.25 | 5.49±1.4 | 5.71±1.6 | <0.01 |
| | expt | (ipsi) | 6.93±1.7 | 7.28±1.8 | 7.51±1.8 | <0.0001 |
| | | (contra) | 5.01±1.3 | 4.48±1.3 | 4.10±1.3 | <0.0001 |
| **ANOVA** | | *p* | <0.0001 | <0.0001 | <0.0001 | |

Experimental mice received monaural middle ear destruction at 10 days after birth and were sacrificed for histology at 15, 30 and 45 days afterwards. Two experimental (expt) and 2 control mice were used for each study session. Data for the control mice were averaged from both ICs. *: mean number of inferior collicular (IC) neurons per each histological slide containing 12 brain sections. **: averaged from 480 neurons. ipsi or contra: the IC ipsilateral or contralateral to the operated ear.Repeated measures one-way ANOVA shows significant difference in number and size of collicular neurons between the control and experimental mice ($p<0.0001$). A Student-Newman-Keuls Multiple Comparison post test shows significant difference between each paired values ($p<0.001$).

pps, unless otherwise stated) by a homemade tone burst generator (electronic switch) driven by a stimulator (Grass S88). The tone pulses were then amplified after passing through a decade attenuator (HP 350D) before they were fed to a small tweeter loudspeaker (surface mount tweeter 40-1217, 4 cm diameter, Radio Shack) that was placed 26.5 cm away from the mouse and 40° contralateral to the recording site. The loudspeaker was calibrated with a Brüel and Kjaer 1/2 inch microphone (4134) placed at the mouse's ear. Output was expressed in dB SPL referred to 20 μPa root mean square. A frequency characteristics curve was plotted for the loudspeaker to determine the maximal available stimulus intensity at each frequency. The frequency characteristics curve was more or less flat for frequency up to 30 kHz but dropped of sharply at higher frequencies.

Upon isolation of an IC neuron with 4 ms pure tone stimuli, the frequency and intensity of the sound stimulus were systematically varied to determine the best excitatory frequency (BF) at which the neuron had the lowest threshold to sound stimulus (i.e. the MT). At the MT, the neuron responded to each of two consecutive presentations of BF stimuli. The neuron's frequency tuning curve (FTC) was measured by determining the threshold of each responsive frequency.

Recorded action potentials were amplified, band-pass filtered (Krohn-Hite 3500), and fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway 2000, 486) for acquisition of peri-stimulus-time (PST) histograms (bin width: 500 μs, sampling period: 300 ms) to 16 stimulus presentations. The PST

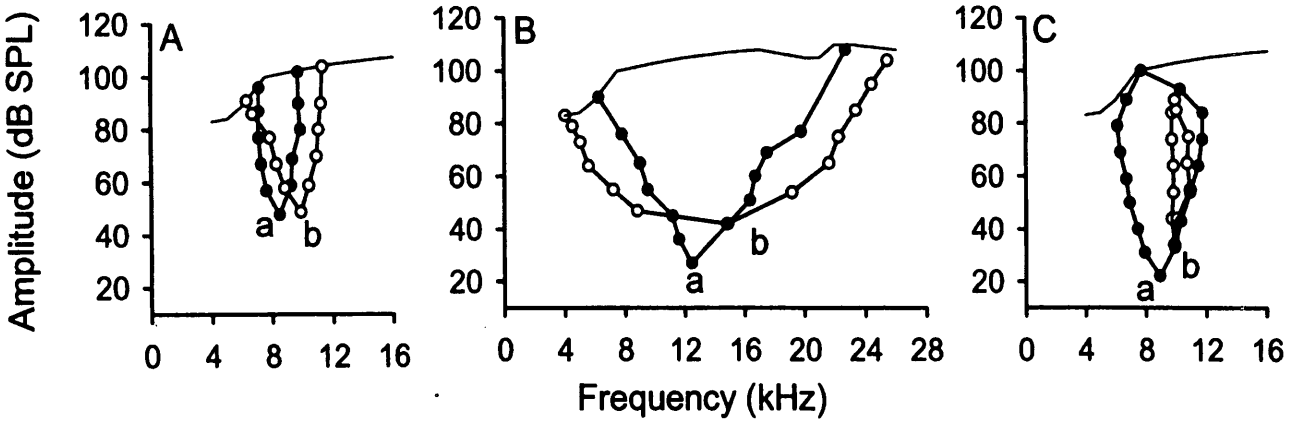


Fig. 3. Representative narrow (A), broad (B) and closed (C) frequency tuning curves (FTCs) of 6 representative IC neurons. The ordinate and abscissa represent stimulus amplitude (dB SPL) and frequency (kHz). The upper solid line represents the frequency characteristics of the loudspeaker. The BF (kHz), MT (dB SPL), recording depth (μm) and latency of these neurons were 8.5, 48, 220, 21 (Aa); 10.0, 24, 1010, 7 (Ab); 12.6, 27, 330, 21 (Ba); 14.8, 42, 620, 15 (Bb); 8.9, 22, 560, 12 (Ca); 9.9, 34, 600, 16 (Cb) (see text for details).

**Table 2. Distribution of Discharge Patterns of Inferior Collicular Neurons from Control and Experimental**

| Discharge pattern | Control | Experimental | |
|---|---|---|---|
| | | ipsi | contra |
| Tonic responders | 25(20%) | 26(22%) | 12(17%) |
| Phasic responders | 62(49%) | 56(48%) | 34(49%) |
| Phasic bursters | 39(31%) | 35(30%) | 24(34%) |

All recordings were made 4 weeks after monaural middle ear destruction. Data for the control mice were obtained from both ICs. A statistical analysis reveals significant different distributions of these three types of responders for neurons between contralateral IC of experimental mice and control mice as well as between two ICs of experimental mice ($\chi^2$ test, $p<0.05$).

**Table 3. Distribution of Frequency Tuning Curves of Inferior Collicular Neurons from Control and Experimental Mice**

| Type | Control | Experimental | |
|---|---|---|---|
| | | ipsi | contra |
| Narrow | 28(23%) | 27(21%) | 27(28%) |
| Broad | 89(73%) | 99(77%) | 69(70%) |
| Closed | 5(4%) | 2(2%) | 2(2%) |

A statistical analysis reveals significant different distributions of these three types of FTCs for neurons between contralateral IC of experimental mice and control mice as well as between two ICs of experimental mice ($\chi^2$ test, $p<0.05$). See Table 2 for legends.

histograms quantitatively describe the discharge pattern of each neuron obtained under different stimulation conditions.

The neuron's latency was determined as the time lag between onset of the stimulus and peak response in the PST histogram obtained at 10 dB above the MT. The sharpness of FTCs was expressed by $Q_n$ ($Q_{10}$, $Q_{30}$) values which were obtained by dividing the BF by the bandwidths of the FTC at 10 and 30 dB above the MT. Data obtained from control and experimental mice were then statistically compared using t test or chi-square test.

## Results

### *The Density, Number and Size of IC Neurons of Control and Experimental Mice*

Monaural middle ear destruction produced a profound effect on postnatal development of IC neurons in experimental mice. The ipsilateral IC of experimental mice always had significantly larger size of IC neurons than the contralateral IC had. Fig. 1 shows the

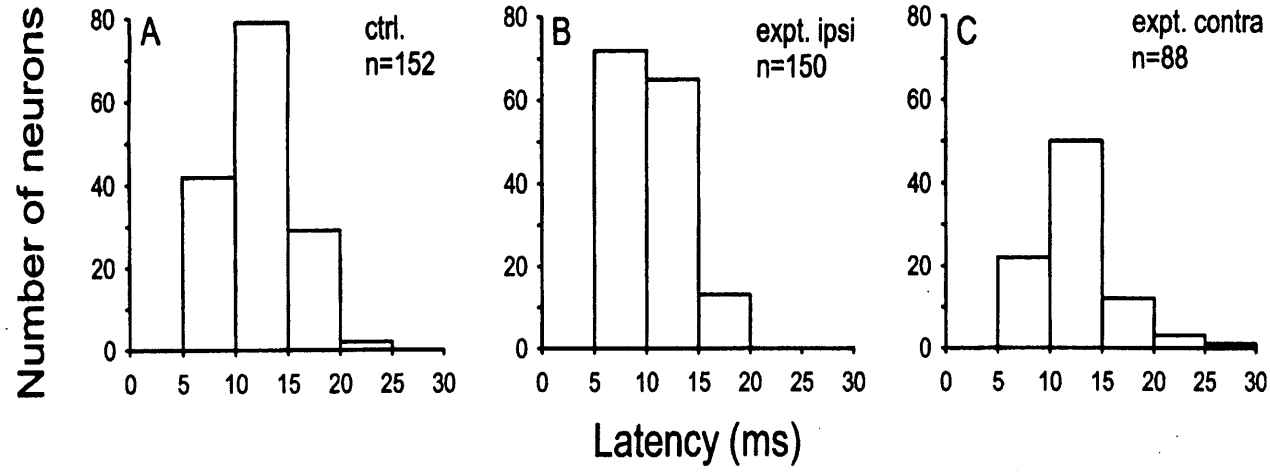


Fig. 4. Latency distribution of IC neurons of control mice (A: ctrl.) and experimental (expt, B, C) mice. Ordinates and abscissae represent number of neurons and latency (ms). n: total number of IC neurons. ipsi or contra: collicular neurons recorded from the IC ipsilateral or contralateral to the ear with middle ear destruction.

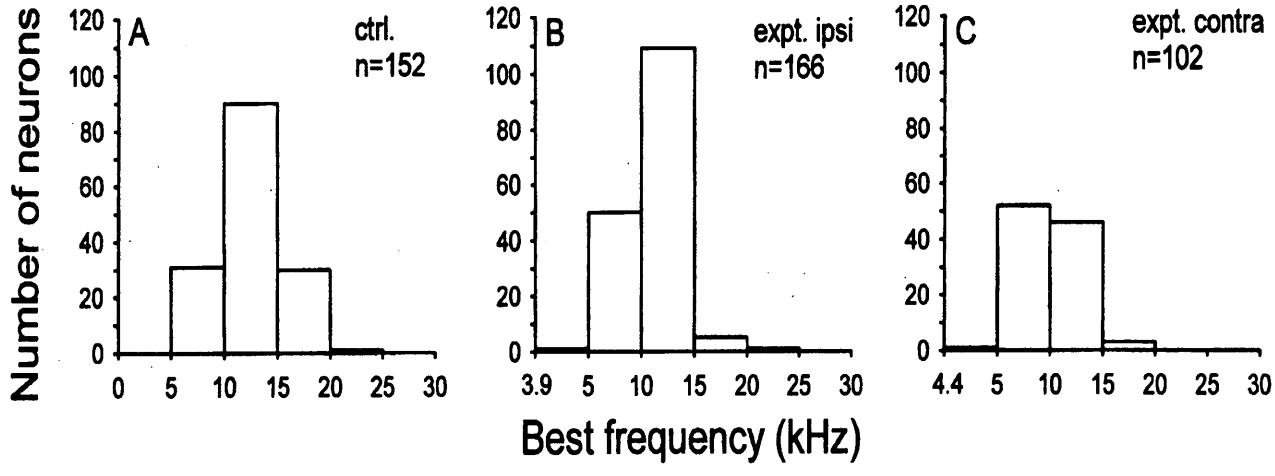


Fig. 5. BF distribution of IC neurons of control mice (A: ctrl.) and experimental (expt, B, C) mice. Ordinates and abscissae represent number of neurons and BF (kHz)(see Fig. 4 for legends).

photomicrographs of cresyl violet staining of IC neurons from control mice and experimental mice examined at age of 58 days after birth. In comparison with the neurons of control mice (Fig. 1A), neurons in the ipsilateral IC of experimental mice had the largest soma size (Fig. 1B) and neurons in the contralateral IC had the smallest soma size (Fig. 1C).

The density, number and soma size of IC neurons of both control and experimental mice determined at different postnatal periods are summarized in Table 1. The density varied within 3% for IC neurons in control mice and the ipsilateral IC of experimental mice but varied more than 40% for neurons in the contralateral IC of experimental mice (Table 1 upper row). The number of IC neurons of both groups of mice significantly varied with age (Table 1 middle row). Variation was less than 1-3 % for neurons in control mice and in the ipsilateral IC of experimental mice but was greater than 24-56% in the contralateral IC of experimental mice. The number of IC neurons in the control mice progressively increased with age but the opposite was observed for neurons in the contralateral IC of experimental mice. When examined at each postnatal period, the contralateral IC of experimental mice had significantly smaller number of IC neurons than the ipsilateral IC of experimental mice and the IC of control mice had (One-way ANOVA, $p<0.0001$).

The size of IC neurons in both groups of mice also varied with age. Variation was less than 4.8% for neurons in control mice and 8.3% for neurons in the ipsilateral IC of experimental mice but was 18% for neurons in the contralateral IC of experimental mice

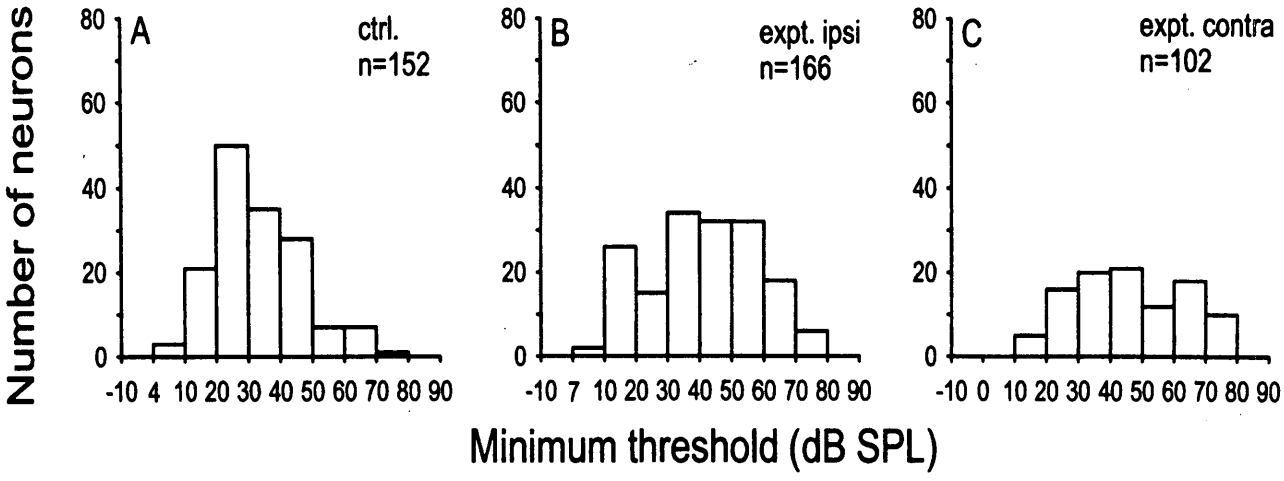


Fig. 6. MT distribution of IC neurons of control mice (A: ctrl.) and experimental (expt, B, C) mice. Ordinates and abscissae represent number of neurons and MT (dB SPL)(see Fig. 4 for legends).

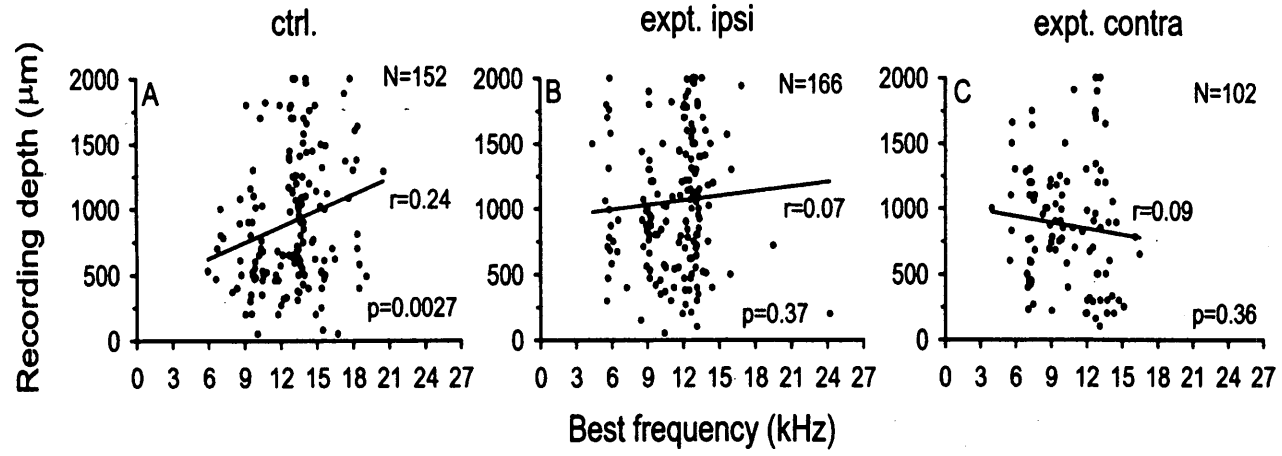


Fig. 7. Scatter plots showing the relationship between BFs (kHz, abscissa) and recording depth (μm, ordinate) of IC neurons from control and experimental mice. The linear regression line and correlation coefficient for each plot are shown by a solid line and r. p: significance level. N: total number of neurons. Note that clear tonotopic organization is not observed fin either IC of experimental mice (B, C).

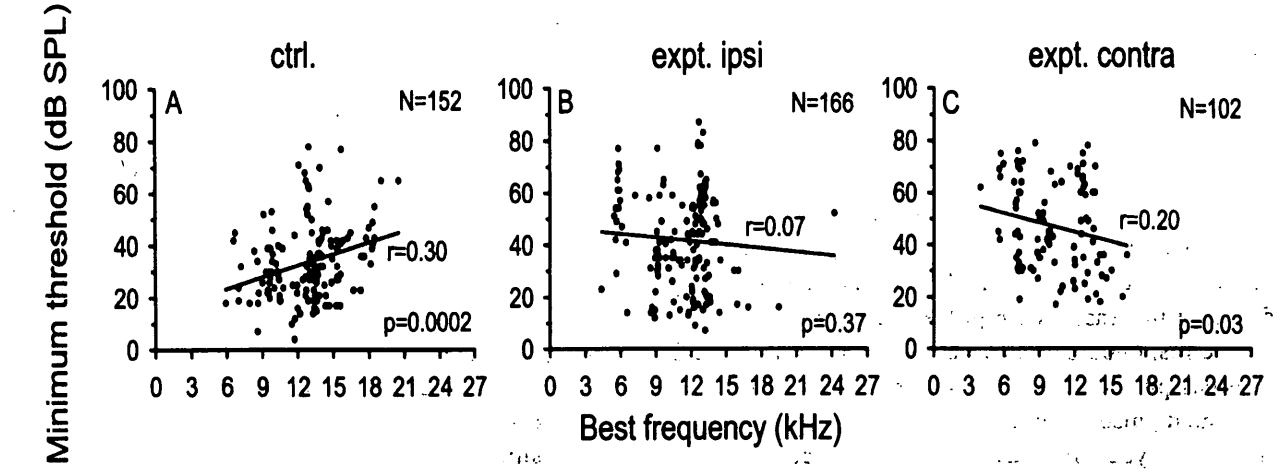


Fig. 8. Scatter plots showing the relationship between BFs (kHz, abscissa) and MTs (dB SPL, ordinate) of IC neurons from control and experimental mice. Note that a clear relationship between MT and BF is only observed for IC neurons of control mice.

**Table 4. Comparisons of Sharpness of Frequency Tuning Curves of Inferior Collicular Neurons from Control and Experimental Mice**

| $Q_n$ values | | Control | Experimental | t test *p* |
|---|---|---|---|---|
| $Q_{10}$ | | | | |
| ipsi | range | 1.3-13.9 (122) | 0.6-13.0 (128) | |
| | m±sd | 4.7±3.6 | 3.9±2.5 | <0.01 |
| contra | range | | 1.0-15.7 (98) | |
| | m±sd | | 3.5±2.5 | <0.05 |
| t test *p* | | | >0.05 | |
| $Q_{30}$ | | | | |
| ipsi | range | 0.7-11.0 (92) | 0.6-5.5 (52) | |
| | m±sd | 2.0±1.5 | 1.7±0.9 | >0.05 |
| contra | range | | 0.9-3.3 (25) | |
| | m±sd | | 1.7±0.8 | >0.05 |
| t test *p* | | | >0.05 | |

See Table 2 for legends.

(Table 1 third row). The size of IC neurons in control and ipsilateral IC of experimental mice significantly increased with age but the opposite was observed for neurons in the contralateral IC of experimental mice (one-way ANOVA, $p<0.01$-$0.0001$). When determined at all ages, the ipsilateral IC of experimental mice had the largest neurons and the contralateral IC have the smallest neurons. Neurons in the IC of control mice had the intermediate size. The size of IC neurons in the control and in both ICs of experimental mice differed significantly at all ages determined (One-way ANOVA, $p<0.0001$).

*Discharge Pattern*

The discharge patterns of 313 IC neurons in response to presented pulses can be described as tonic responders, phasic responders and phasic bursters. Tonic responders discharged impulses throughout or longer than the duration of presented pulses and their discharge duration increased with pulse duration (Fig. 2A,B). Phasic responders discharged 1-2 impulses (Fig. 2C) whereas phasic bursters discharged 3-5 impulses to presented pulses (Fig. 2D). The discharge duration of these two types of neurons was not affected by pulse duration. As shown in Table 2, most (78-83%) IC neurons were either phasic responders or phasic bursters (Table 2 middle and bottom rows). However, the ipsilateral IC of experimental mice had a larger percent of tonic responders than the contralateral IC (Table 2 top row). The distribution of discharge patterns in the ipsilateral IC of experimental mice was within 2% of the IC of control mice. A statistical analysis reveals significant different distributions of these three types of responders for neurons between contralateral IC of experimental mice and two Ics of control mice as well as between two ICs of experimental mice ($\chi^2$ test, $p<0.05$).

*FTCs*

FTCs of all IC neurons were either V-shaped or closed (upper threshold). At the strongest intensity, V-shaped FTCs had different band-widths. For convenience, we describe V-shaped FTCs with bandwidths less than 10 kHz at the strongest intensity as narrow (Fig. 3A) and those with bandwidths greater than 10 kHz as broad (Fig. 3B). Narrow FTCs had sharper slopes at both flanks (Fig. 3A) and broad FTCs had shallow slopes (Fig. 3B). Upper threshold or closed FTCs had thresholds at both low and high stimulus intensities. The ascending flanks from the lowest MT widened to a certain degree before closing at highest stimulus intensity (Fig. 3C).

**Table 5. A Comparison of Response Latency, Best Frequency and Minimum Threshold of Inferior Collicular Neurons from Control and Experimental Mice**

| Response properties | | Control | Experimental | t test *p* |
|---|---|---|---|---|
| **Latency (ms)** | | | | |
| **ipsi** | **range** | 7-22 (152) | 5-18 (150) | |
| | **m±sd** | 12.7±3.3 | 11.0±2.9 | <0.001 |
| **contra** | **range** | | 7-29 (88) | |
| | **m±sd** | | 13.3±3.7 | >0.05 |
| **t test *p*** | | | <0.001 | |
| **BF (kHz)** | | | | |
| **ipsi** | **range** | 5.9-20.5 (152) | 4.4-24.2 (166) | |
| | **m±sd** | 12.9±2.9 | 11.3±2.9 | <0.001 |
| **contra** | **range** | | 3.9-16.4 (102) | |
| | **m±sd** | | 10.2±2.9 | <0.001 |
| **t test *p*** | | | <0.001 | |
| **MT (dB SPL)** | | | | |
| **ipsi** | **range** | 4-78 (152) | 7-87 (166) | |
| | **m±sd** | 33.6±13.6 | 41.8±17.6 | <0.001 |
| **contra** | **range** | | 17-79 | |
| | **m±sd** | | 46.9±17.1 (102) | <0.001 |
| **t test *p*** | | | >0.05 | |

See Table 2 for legends.

Most (70-77%) IC neurons had broad FTCs and only a few (less than 4%) had closed FTCs (Table 3). Collicular neurons in the ipsilateral IC of experimental mice had more broad FTCs and fewer narrow FTCs than neurons in the contralateral IC and in the control mice. A statistical analysis reveals significant different distribution of these three types of FTCs for neurons between contralateral IC of experimental mice and two ICs of control mice as well as between two ICs of experimental mice ($\chi^2$ test, $p<0.05$).

A comparison of sharpness (in $Q_n$ values) of FTCs of IC neurons from both groups of mice is shown in Table 4. The average $Q_{10}$ value was significantly larger for IC neurons in control mice than in either IC of experimental mice (t test, $p< 0.01$-$0.05$). However, average $Q_{30}$ values of IC neurons among control and experimental mice were not significantly different (t test, $p>0.05$). Significant differences were also not found for both $Q_{10}$ and $Q_{30}$ values of neurons in the two ICs of experimental mice (t test, $p>0.05$).

### *Response Latency, BF and MT*

Response latencies of all IC neurons to BF sounds delivered at 10 dB above the MT were between 5 and 29 ms. Most latencies of IC neurons in control mice and contralateral IC of experimental mice were between 10 and 15 ms (Fig. 4A, 4C). However, they were between 5 and 15 ms in the ipsilateral IC of experimental mice (Fig. 4B).

IC neurons of control mice had a significantly longer average latency than neurons in the ipsilateral IC of experimental mice (t test $p<0.001$, Table 5 top row). For experimental mice, the average latency was significantly longer for neurons in the contralateral IC

than in the ipsilateral IC (t test, $p<0.001$).

BFs of all IC neurons were between 4.4 and 24.2 kHz. While most BFs were between 10 and 15 kHz for neurons in control mice and ipsilateral IC of experimental mice, they were between 5 and 15 kHz in the contralateral IC of experimental mice (Fig 5A, 5B vs 5C). The average BF was significantly higher for neurons in the IC of control mice than for neurons in both ICs of experimental mice (Table 5 middle row, t test, $p<0.001$). The average BF was also significantly higher for neurons in the ipsilateral IC than in the contralateral IC of experimental mice (t test, $p<0.001$).

MTs of all IC neurons were between 4 and 87 dB SPL. Most MTs were between 10 and 50 dB SPL for IC neurons in control mice but were broadly distributed between 10 and 70 dB SPL for IC neurons in experimental mice (Fig. 6A vs 6B, 6C). The average MT of IC neurons was significantly lower for control mice than for experimental mice (Table 5 bottom row, t test, $p<0.001$). However, the average MT of neurons in both ICs of experimental mice was not significantly different (t test, $p>0.05$).

### *Tonotopic Organization and Relationship between MT and BF*

Tonotopic organization of neurons in the IC of both groups of mice was examined by linear regression analyses of scatter plots of recording depths of IC neurons in relation to BFs. A significant correlation was observed between the recording depth and BF for IC neurons in control mice (Fig. 7A, $p<0.005$) but not for IC neurons in experimental mice (Fig. 7B, 7C, $p>0.1$). In other words, tonotopic organization was not observed for neurons in either IC of experimental mice.

Figure 8A-C show the scatter plots of MTs of IC neurons in both groups of mice in relation to BFs. Linear regression analyses only revealed a significant correlation between MT and BF for IC neurons in control mice such that MTs of IC neurons increased with BFs (Fig. 8A, $p<0.001$). However, this correlation was not observed for neurons in either IC of experimental mice (Fig. 8B, 8C, $p>0.01$).

## Discussion

In this study, we examined the effect of monaural middle ear destruction on postnatal development of mouse IC neurons. By removing the tympanic membrane and the ossicular chain monaurally, we produced an asymmetrical sound stimulation condition in which sound intensity to the operated ear was severely attenuated (33,35,36). Under this asymmetrical sound stimulation condition, sound stimulation was strongest to the ipsilateral IC and weakest to the contralateral IC. This difference in excitation between the two ICs could be responsible for the development of large neurons in the ipsilateral IC but small neurons in the contralateral IC of experimental mice (Table 1). A similar finding has been reported for neurons in the IC of neonatally deafened cats (20).

On the other hand, this different size of neurons between two ICs of experimental mice could be due to arrest of growth of neurons in the contralateral IC which would not occur with normal binaural stimulation conditions during the course of development (12). It is conceivable that axon terminals of IC neurons contralateral to the operated ear could be at a competitive disadvantage under asymmetrical sound stimulation conditions.

We also observed that the size of IC neurons in the experimental mice progressively increased in the ipsilateral IC but decreased in the contralateral IC with postnatal developmental age (Table 1). This observation suggests that the sensitive period for this mouse species may last at least 45 DAB. This is consistent with a previous report that the basic circuitry of the auditory system appears to be established by the time of onset hearing, but changes in dendritic and axonal morphology continue over a protracted time period, well beyond the onset of hearing and probably into adulthood (18).

We have shown that neurons in the contralateral IC of experimental mice typically had longer latencies, lower BFs, and higher MTs than neurons in the ipsilateral IC (Table 5). However, monaural middle ear destruction appears to produce less effect on response properties of IC neurons of experimental mice than unilateral cochlear ablation. Previous studies have shown that cochlear removal produces a threefold increase in the number of excitatory response neurons with lower threshold and shorter latency in the ipsilateral IC (3,4,16,17). This different effect is likely due to the fact that monaural middle ear destruction only modifies acoustic inputs to the auditory system while cochlear ablation reduces receptors and neuronal activity as well as produced degeneration or atrophy in the auditory nerve and auditory nuclei (4,31). For this reason, the difference in excitation between ipsilateral and contralateral ICs would be greater in mice with monaural cochlear

ablation than in mice with monaural middle ear destruction.

Previous studies showed that most IC neurons are mainly excited contralaterally and inhibited ipsilaterally (i.e. EI neurons) while some are excited bilaterally (i.e. EE neurons) or contralaterally, only (i.e. EO neurons) (6,14,30). In this study, sound stimulation was strongest to the ipsilateral IC but weakest to the contralateral IC of experimental mice. For this reason, EI neurons contralateral to the intact ear received stronger excitation from the unoperated ear relative to attenuated inhibition from the operated ear. In contrast, EI neurons ipsilateral to the intact ear received weaker excitation from the operated ear relative to unattenuated inhibition from the unoperated ear. By the same token, EE or EO neurons contralateral to the intact ear received stronger excitation from the unoperated ear than EE or EO neurons ipsilateral to the intact ear. Thus, the ipsilateral IC of experimental mice would receive more excitation from the unoperated ear and the contralateral IC would receive less excitation from the operated ear regardless of the aurality of IC neurons. These varying degrees of excitation could be responsible for the development of larger soma size, shorter latencies, lower MTs and more broad FTCs for neurons in the ipsilateral IC than in the contralateral IC of experimental mice (Fig. 1; Tables 1,3,5). However, monaural middle ear destruction apparently disrupted the normal balance between excitation and inhibition to both ICs so that MTs of IC neurons were significantly higher in experimental mice than in control mice (Table 5 bottom row). This imbalance of neural inputs between two ICs would also severely impair the ability of sound localization of the experimental mice. For example, neurons in the contralateral IC of the control mice are typically more sensitive to contralateral than ipsilateral sounds but the opposite was observed for neurons in the contralateral IC of the experimental mice (L. J. Xu and P. H.-S. Jen, unpublished observations).

Previous studies have shown that IC neurons of mice may have BFs as high as 50 kHz (13,38). However, because of the frequency characteristics of the loudspeaker which dropped off sharply at frequency higher than 30 kHz, we only recorded IC neurons with BFs ranged between 4.4 and 24.2 kHz (Table 5). In spite of this limitation, clear tonotopic organization was observed for neurons in the IC of control mice but not for neurons in either IC of experimental mice (Fig. 7A vs 7B, 7C). This finding suggests that symmetrical sound stimulation is essential for postnatal development of tonotopic organization in experimental mice. Significant correlation between BFs and MTs was observed for IC neurons in control mice but not in experimental mice (Fig. 8). This finding might be due to the fact that monaural middle ear destruction significantly raised MTs of IC neurons of experimental mice relative to control mice (Table 5 bottom row). This change in MTs of IC neurons redistributed the scatter plots which results in the disruption of the correlation between MT and BF of IC neurons.

In summary, we found that monaural middle ear destruction produced a greater effect on the number and size of neurons in contralateral than in the ipsilateral IC of experimental mice. This effect increased with increasing postoperative survival periods. Monaural middle ear destruction also produced significantly longer latencies, higher minimum thresholds, and smaller dynamic ranges for neurons in contralateral IC than in ipsilateral IC relative to the destructed ear. Monaural destruction also disrupted the postnatal development of tonotopic organization in the IC of experimental mice.

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