# The Effect of Bicuculline Application on Auditory Response Properties of Inferior Collicular Neurons of Mice With or Without Monaural Middle Ear Destruction in Early Age

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## Abstract

**Previous studies have demonstrated that abnormal auditory stimulation during early postnatal development can be manifested through physiological changes that occur in the inferior colliculus (IC) of mammals. To determine the contribution of the GABAergic transmitter systems to the development of response properties of IC neurons, we examined the effect of application of bicuculline (which is an antagonist for the $GABA_A$ receptors) on response properties of IC neurons of the laboratory mice, *Mus musculus*, with or without early monaural middle ear destruction. Monaural middle ear destruction was performed at 12-14 days after birth. At adulthood, the auditory response properties of IC neurons were examined in both experimental conditions. All IC neurons determined before and during bicuculline application can be described as (1) phasic responders which discharged 1-2 impulses; (2) phasic bursters which discharged 3-7 impulse; and (3) tonic responders which discharged impulses throughout the duration of presented sound pulses. Early monaural middle ear destruction only affected the percent distribution but not the type of discharge pattern, rate-intensity function and frequency tuning curve of IC neurons in the control and experimental mice. Neurons in the contralateral IC of experimental mice typically had longer latencies, higher minimum thresholds, broader frequency tuning curves and smaller dynamic ranges than neurons in the ipsilateral IC and in control mice. Bicuculline application produced differential effects in decreasing the latencies and minimum thresholds as well as broadening frequency tuning curves and dynamic ranges of IC neurons in these two groups of mice. All these data suggest that early monaural middle ear destruction did not affect the shaping of auditory response properties of IC neurons by GABAergic transmitter system.**

**Key Words: bicuculline, frequency tuning curve, middle ear destruction, mouse, rate-intensity function**

## Introduction

Previous studies have shown that monaural plugging permanently changes the auditory spatial sensitivity of neurons in the inferior colliculus (IC) of bats (19) and produces substantial loss of binaural interaction in the IC of rats (9, 45). Unilateral cochlear removal produces a threefold increase in the number of excitatory response neurons in the ipsilaterally excited IC recording loci with lower thresholds, wider dynamic ranges, more sustained discharge patterns and shorter latencies (1,22,23,28). Conversely, early stimulation

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with a specific sound frequency results in manifestation of IC neurons tuned to the early experienced sound frequency in mice (40) and rats (38,39). Anatomical studies showed that monaural deprivation or cochlear ablation led to a decrease in soma size and number in the IC of mice (52,53), rats (21), cats (14,15,33), gerbils (16,27), and ferrets (29,30).

Our previous study (56) has shown that early monaural middle-ear destruction produces larger neurons in the adult ipsilateral IC. In addition, it significantly reduces the number and distribution density of neurons in the contralateral IC. In another study, we found that IC neurons of control mice typically had lower minimum thresholds, larger dynamic ranges and $Q_{10}$ values than IC neurons of experimental mice (54). All these studies clearly demonstrate that early abnormal auditory experience can be manifested through the anatomical and physiological changes that occur in the central auditory system.

Previous studies have shown that inhibitory and excitatory transmitter systems emerge at roughly at the same developmental age in the auditory nuclei (7,42, 43). For example, glutamate and GABA concentrations in the IC of rats increase monotonically and reach a maximum at postnatal day of 21 and then decline and attain adultlike values after the first postnatal month (41). These observations are consistent with immunocytochemical studies which showed a developmental increase in the expression of GABA during the postnatal period (7,42,46). Based upon these studies, we hypothesize that the differences in auditory response properties between IC neurons of mice with and without early monaural middle ear destruction is a result of disruption of normal development of the GABAeric transmitter system. To test this hypothesis, we studied the effect of bicuculline application on the discharge patterns, rate-intensity functions and frequency tuning curves (FTCs) of IC neurons of control and experimental mice.

## Material and Methods

Two groups of CD1, *Mus musculus* (body weight, b.w., 28-34 g) were used for this study. For the control mice (n= 8, 4 males and 4 females), auditory response properties of IC neurons were examined at 6-8 weeks after birth. For the experimental mice (n=10, 6 males and 4 females), one middle ear was destroyed at 12-14 days after birth and recording of IC neurons was conducted at 6-8 weeks after birth.

**Table 1. The Discharge Patterns of Inferior Collicular Neurons of Control and Experimental Mice Determined before and during Bicuculline Application**

| Discharge pattern | Predrug | Bicuculline | | |
|---|---|---|---|---|
| | | p | pb | t |
| Control | | | | |
| p | 14(52%) | 4 | 4 | 6 |
| pb | 10(37%) | 0 | 4 | 6 |
| t | 3(11%) | 0 | 0 | 3 |
| Total | 27 | 4(15%) | 8(30%) | 15(55%) |
| Experimental | | | | |
| Ipsi | | | | |
| p | 13(39%) | 5 | 4 | 4 |
| pb | 11(33%) | 0 | 5 | 6 |
| t | 9(28%) | 0 | 0 | 9 |
| Total | 33 | 5(15%) | 9(27%) | 19(58%) |
| Contra | | | | |
| p | 15(54%) | 6 | 3 | 3 |
| pb | 9(32%) | 0 | 3 | 6 |
| t | 4(14%) | 0 | 0 | 4 |
| Total | 28 | 6(21%) | 6(21%) | 16(58%) |

Experimental mice received monaural middle ear destruction at 12-14 days after birth. All recordings were made at least 4 weeks after monaural middle ear destruction. Numbers underlined indicate no change in discharge pattern. p: phasic responder, pb: phasic burster;t: tonic responder. Ipsi or contra: IC neurons ipsilateral or contralateral to the operated ear.

Monaural middle ear destruction was performed under Nembutal anesthesia (50 mg/kg b.w.). A pair of fine forceps was inserted through the ear canal under the light microscope to remove the tympanic membrane and the ossicular chain. 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 (47,49,50).

The surgery procedures for recording were basically the same as in a previous study (Cain and Jen 1999). Briefly, each mouse 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 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 (48). Glass electrodes of 3M KCl (impedance: 2-5 MΩ) were

**Table 2. Percent Increase in the Maximal Number of Impulses of Inferior Collicular Neurons of Control and Experimental Mice during Bicuculline Application**

| % increase | Control | Experimental | |
|---|---|---|---|
| | | Ipsi | Contra |
| <10% | 0(0%) | 3(9%) | 0(0%) |
| 10-100% | 8(30%) | 14(42%) | 10(36%) |
| 100-400% | 15(56%) | 16(49%) | 14(50%) |
| >400% | 4(14%) | 0(0%) | 4(14%) |
| Total | 27 | 33 | 28 |

The percent increase was obtained by dividing the increase in the maximal number of impulses during bicuculline application by the pre-drug maximal number of impulses.

inserted into the IC to record sound-evoked neural responses. Recording depth was read from the scale of a microdrive (David-Kopf). Additional doses (10 mg/kg) of Nembutal were administered during later phases of recording when necessary. An indifferent electrode was placed at the nearby temporal muscles. Each mouse was used in one to 6 recording sessions on separate days and each recording session typically last 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 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, 4cm 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. The 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.

When an IC neuron responding to 4 ms sounds was isolated, the frequency and intensity of the sound were systematically varied to determine the best excitatory frequency (BF) at which the neuron had the lowest threshold to sound stimulus (i.e. the minimum threshold or MT). At the MT, the neuron responded to each of two consecutive presentations of BF pulses. The neuron's FTC was measured by determining the threshold of each responsive frequency. The neuron's rate-intensity function was then studied by recording the number of impulses to BF sounds delivered at 10 dB increments above the MT. These numbers were

**Table 3. A Comparison of Response Latency, and Minimum Threshold of Inferior Collicular Neurons of Control and Experimental Mice**

| Response properties | | Control | Experimental | t-test p |
|---|---|---|---|---|
| Latency (ms) | | | | |
| Ipsi | n | 27 | 33 | |
| | Range | 7-17 | 8-17 | |
| | Mean±sd | *10.9±2.5 | 11.8±2.3 | 0.1524 |
| Contra | n | 27 | 28 | |
| | Range | 7-17 | 10-19 | |
| | Mean±sd | *10.9±2.5 | 13.3±2.4 | 0.0004 |
| t-test, p | | | <0.05 | |
| MT (dB SPL) | | | | |
| Ipsi | n | 27 | 33 | |
| | Range | 7-78 | 7-87 | |
| | Mean±sd | *40.0±18.7 | 41.1±21.4 | 0.8348 |
| Contra | n | 27 | 28 | |
| | Range | 7-78 | 30-79 | |
| | Mean±sd | *40.0±18.7 | 50.8±16.1 | 0.0194 |
| t test, p | | | <0.05 | |

*Average values from neurons of both inferior colliculi. For convenience of comparison, the average value is listed twice.

then used to plot against the stimulus intensity. To determine the contribution of GABAergic inhibition on auditory response properties of IC neurons, the discharge pattern, response latency, FTC, and rate-intensity function of IC neurons of control and experimental mice were examined before and during application of bicuculline which is an antagonist for $GABA_A$ receptors (2,8).

The construction of the piggy-back multibarrel electrodes and iontophoretical injection of bicuculline have been described in detail in previous studies (24, 25). Briefly, a three-barrel electrode (tip: 10-15 μm) was "piggybacked" to a 3 M KCl single-barrel electrode (tip: less than 1 μm; impedance: 5-10 MΩ whose tip was extended about 10 μm from the tip of the three-barrel electrode. The 3 M KCl single-barrel electrode was used to record neural response. One of the barrels of a three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 M NaCl, pH 3.0) for injection into the recording site. The bicuculline was prepared just prior to each experiment and the electrode filled immediately before use. This bicuculline channel was connected via silver-silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) which was used to generate and monitor iontophoretic currents. During bicuculline application, a 1 s pulse of 40 nA at 0.5 pps was applied for 1 min before data acquisition. Application current was then changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground

**Table 4. The Response Latency and Minimum Threshold of Inferior Collicular Neurons of Control and Experimental Mice Determined before and during Bicuculline Application**

| Response properties | | Control Predrug | Bic | Paired t-test, p | Experimental Predrug | Bic | Paired t test, p |
|---|---|---|---|---|---|---|---|
| Latency (ms) | | | | | | | |
| Ipsi | n | 14 | 14 | | 19 | 19 | |
| | Range | *10-15 | *9-14 | | 8-17 | 7-16 | |
| | Mean±sd | *11.7±1.5 | *10.6±1.6 | <0.0001 | 11.8±2.5 | 10.8±2.3 | <0.001 |
| % change | | | 9.4% | | | 8.5% | |
| Contra | n | | | | 19 | 19 | |
| | Range | | | | 10-19 | 9-18 | |
| | Mean±sd | | | | 14.3±2.5 | 13.0±2.8 | <0.001 |
| % change | | | | | | 9.1% | |
| t test, P | | | | | <0.005 | <0.01 | |
| MT (dB SPL) | | | | | | | |
| Ipsi | n | 25 | 25 | | 21 | 21 | |
| | Range | *7-78 | *1-70 | | 7-87 | 5-84 | |
| | Mean±sd | *40.9±19.2 | *32.9±17.6 | <0.0001 | 40.0±23.1 | 36.4±23.1 | <0.0001 |
| % change | | | 19.6% | | | 9% | |
| Contra | n | | | | 25 | 25 | |
| | Range | | | | 30-79 | 24-72 | |
| | Mean±sd | | | | 52.4±16.2 | 47.6±16.2 | <0.0001 |
| % change | | | | | | 9.2% | |
| t test, P | | | | | <0.05 | <0.05 | |

*Average values from neurons of both inferior collicului. % change in latency or MT due to bicuculline application

**Table 5. A Comparison of $Q_{10}$ Values and Dynamic Range of Inferior Collicular Neurons of Control and Experimental Mice**

| Response properties | | Control | Experimental | t-test P |
|---|---|---|---|---|
| $Q_{10}$ values | | | | |
| Ipsi | n | 27 | 33 | |
| | Range | 1.7-13.6 | 0.6-13 | |
| | Mean±sd | *5.3±3.1 | 4.3±3.1 | 0.2188 |
| Contra | n | 27 | 28 | |
| | Range | 1.7-13.6 | 1.4-8.1 | |
| | Mean±sd | *5.3±3.1 | 3.8±2.9 | 0.0582 |
| t-test, P | | | >0.05 | |
| Dynamic range (dB) | | | | |
| Ipsi | n | 27 | 33 | |
| | Range | 11-80 | 9-69 | |
| | Mean±sd | *37.3±23.5 | 33.2±21.7 | 0.4858 |
| Contra | n | 27 | 28 | |
| | Range | 11-80 | 7-55 | |
| | Mean±sd | *37.3±23.5 | 18.5±13.9 | 0.0006 |
| t test, P | | | <0.05 | |

See Table 3 for legends.

and the other as the balanced barrel. The balance electrode was connected to the balance module. The retaining current was negative 8-10 nA. Bicuculline application was considered to have blocked all $GABA_A$ receptors of the neuron when its response monitored at least three times did not vary more than 15% even at higher application current (60 nA).

To assess the stability of the neuron's response, the neuron was allowed to recover from bicuculline application (5 minutes after termination of bicuculline application) and its response properties were remeasured and compared with its predrug response properties. A neuron was discarded if its responses fluctuated drastically during recording. Data were also discarded when the impedance of the bicuculline-filled electrode varied more than 20 MΩ before and after the recording, if the tip of the three-barrel electrode broke when withdrawn from the recording site or when the tips of the single and the three-barrel electrode separated from each other.

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

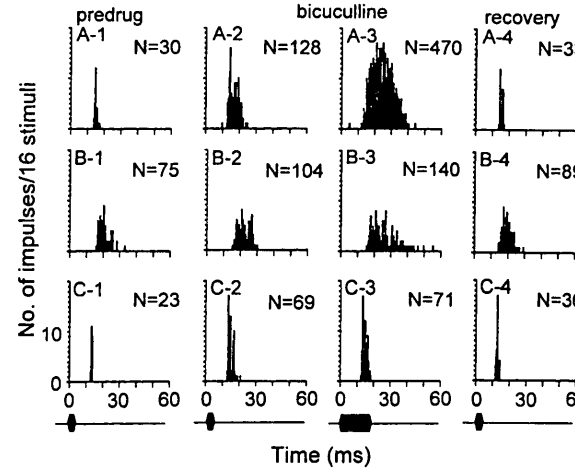


Fig. 1. Peri-stimulus-time histograms (PSTs) of three IC neurons showing the discharge patterns obtained before (predrug), during (bicuculline) and after (recovery) bicuculline application. Bicuculline application changed the discharge pattern of a phasic responder (A-1) and a phasic burster (B-1) into tonic responders (A-2,A-3,B-2,B-3). The application also changed a phasic responder into phasic burster (D-1,D-2,D-3). Note that the discharge pattern of each neuron during bicuculline application was determined with 4 and 20 ms stimuli (envelops shown at the bottom). N: total number of impulses per 16 stimuli. These three neurons were recorded from the IC of a control mouse (A), the contralateral (B) and ipsilateral (C) ICs of an experimental mouse. The BF (kHz), MT (dB SPL), recording depth (μm) and latency (ms) of these neurons before bicuculline application were 13.0, 62, 1250, 12 (A); 9.8, 45, 900, 15 (B); 9.1, 36, 760, 11 (C).

stimulation conditions.

The neuron's latency was determined as the time lag between onset of the stimulus and the peak response in the PST histogram obtained with BF sounds 10 dB

**Table 6. $Q_{10}$ Values of Frequency Tuning Curves and Dynamic Ranges of Inferior Collicular Neurons of Control and Experimental Mice Determined before and during Bicuculline**

| Response properties | | Control Predrug | Bic | Paired t test, p | Experimental Predrug | Bic | Paired t test, P |
|---|---|---|---|---|---|---|---|
| $Q_{10}$ | | | | | | | |
| Ipsi | n | 22 | 22 | | 28 | 28 | |
| | Range | *2.3-13.6 | *1.4-6.1 | | 0.6-13 | 0.1-10.1 | |
| | Mean±sd | *5.9±3.1 | *3.7±2.5 | <0.0001 | 4.6±3.3 | 3.3±2.6 | <0.0001 |
| % change | | | 37% | | | 28% | |
| Contra | n | | | | 20 | 20 | |
| | Range | | | | 1.6-8.1 | 0.8-5.1 | |
| | Mean±sd | | | | 4.0±1.9 | 2.2±1.1 | <0.0001 |
| % change | | | | | | 45% | |
| t test, P | | | | | 0.44 | 0.067 | |
| Dynamic Range (dB) | | | | | | | |
| Ipsi | n | 18 | 18 | | 16 | 16 | |
| | Range | *11-80 | *13-87 | | 9-59 | 13-60 | |
| | Mean±sd | *31.8±23.6 | *44.3±22.4 | <0.0001 | 28.8±19.8 | 49.4±17.0 | <0.005 |
| % change | | | 39.3% | | | 71.5% | |
| Contra | n | | | | 22 | 22 | |
| | Range | | | | 7-55 | 15-58 | |
| | Mean±sd | | | | 17.1±12.2 | 26.8±11.6 | <0.0001 |
| % change | | | | | | 56.7% | |
| t test, P | | | | | <0.05 | <0.0001 | |

*Average values from neurons of both inferior colliculi. % change in latency or MT due to bicuculline application

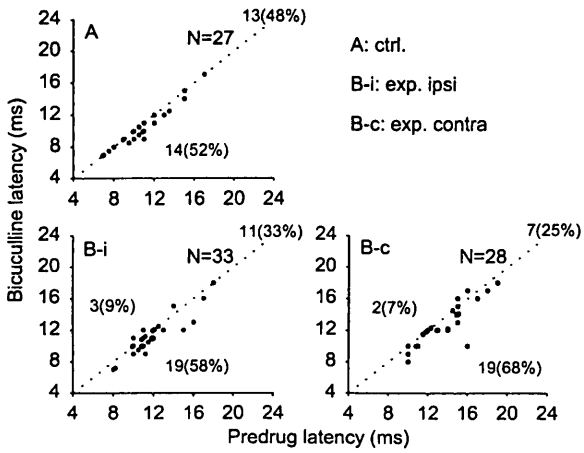


Fig. 2. Comparisons of latencies (ms) of IC neurons obtained before (predrug) and during bicuculline application. Diagonal dotted lines represent equal latency. A: ctrl: Data obtained from IC neurons of control mice. B-i: exp.ipsi, B-c: exp. contra: data obtained from the IC ipsilateral or contralateral to the ear with middle ear destruction. N: number of IC neurons. All data were obtained at adulthood.

above the MT. The sharpness of FTCs was expressed by $Q_{10}$ values which were obtained by dividing the BF by the bandwidths at 10 dB above the MT. A Student T test was used to statistically compare data obtained from IC neurons of control and experimental mice.

## Results

### *The Effect of Bicuculline Application on Discharge Pattern*

**Table 7. The Rate-Intensity Function of Inferior Collicular Neurons of Control and Experimental Mice Determined before and during Bicuculline Application**

| Type | Control Predrug | Bic | Experimental Predrug | Bic |
|---|---|---|---|---|
| Monotonic | | | | |
| Ipsi | *6(22%) | *16(59%) | 9(27%) | 20(61%) |
| Contra | | | 6(22%) | 16 (57%) |
| Nonmonotonic | | | | |
| Ipsi | *21(78%) | *11(41%) | 24(73%) | 13(39%) |
| Contra | | | 22(78%) | 12(43%) |

*: obtained from neurons of both inferior colliculi.

Auditory responses of 27 IC neurons were recorded from control mice, 33 from the ipsilateral IC and 28 from the contralateral IC of experimental mice. Discharge patterns of all IC neurons determined before and during bicuculline application can be described as phasic responders, phasic bursters and tonic responders. Phasic responders discharged 1-2 impulses (Fig. 1A-1, A-4,C-1,C-4) whereas phasic bursters typically discharged 3-7 impulses (Fig. 1B-1,B-4,C-2,C-3). In contrast, tonic responders discharged impulses throughout or longer than the duration of presented pulses (Fig. 1A-2,A-3; B-2,B-3).

Bicuculline application increased the number of impulses of all IC neurons and changed the discharge patterns of 43-60% of IC neurons. As shown in Fig. 1, bicuculline application changed one representative phasic neuron into a tonic responder in which the discharge duration increased with stimulus duration (Fig. 1A-1 vs A-2, A-3). This neuron changed back to a phasic responder after recovery from bicuculline application (Fig. 1A-4). Bicuculline application changed another phasic burster into a tonic responder (Fig. B-1 vs B-2, B-3) which changed back to phasic burster after bicuculline application (Fig. 1B-4). Bicuculline application increased the discharge rate of another phasic neuron but did not change its discharge pattern (Fig. 1C-1,C-2,C-3,C-4).

Table 1 shows the distribution of discharge patterns of IC neurons from both groups of mice determined before and during bicuculline application. Most (72-89%) IC neurons were either phasic responders or phasic bursters before bicuculline application. The ipsilateral IC of experimental mice had twice as many tonic responders than the contralateral IC and the IC of control mice. More than half of IC neurons from both groups of mice were tonic responders during bicuculline application. Bicuculline application did not affect the discharge patterns of 40-57% of IC neurons (numbers underlined).

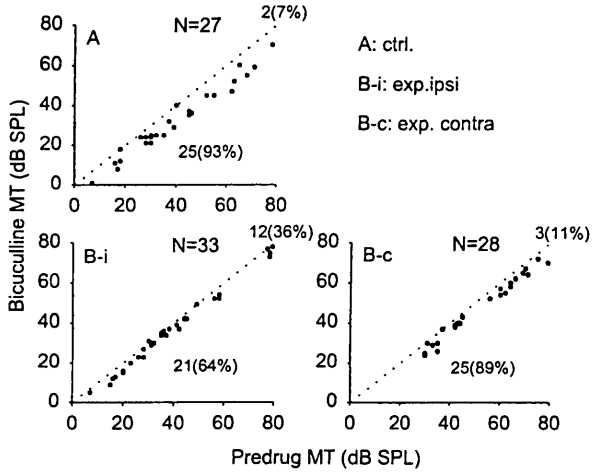


Fig. 3 Comparisons of MTs (dB SPL) of IC neurons obtained before (predrug) and during bicuculline application (See Fig. 2 for legends).

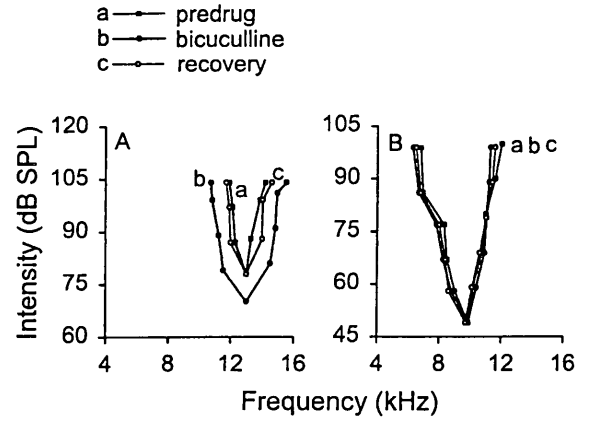


Fig. 4. Frequency tuning curves of two representative IC neurons determined before (solid squares), during (solid circles) and after (unfilled circles) bicuculline application. Aa, Ba: predrug, Ab, Bb: bicuculline application, Ac, Bc: recovery from bicuculline application. These two neurons were recorded from the IC of a control mouse (A) and the ipsilateral IC (B) of an experimental mouse. The BF (kHz), MT (dB SPL), recording depth (μm) and latency (ms) of these two neurons before bicuculline application were 13.0, 78, 1130, 15 (A). 9.9, 49, 1110, 12 (B).

We calculated the percent increase in the maximal discharge by dividing the increase in the maximal number of impulses during bicuculline application by the predrug maximal number of impulses. As shown in Table 2, the percent increase in the maximal number of impulses in most (86-91%) IC neurons during bicuculline application was between 10% and 400%. A few neurons in control mice and in the contralateral IC of experimental mice had an increase in the maximal number of impulses greater than 400% but none had less than 10%. In contrast, a few neurons in the ipsilateral IC of experimental mice had an increase in the maximal number of impulses less than 10% but none had greater than 400%.

### *The Effect of Bicuculline Application on Latency and MT*

When measured before bicuculline application, neurons in the contralateral IC of experimental mice had significantly longer average latencies than neurons in the ipsilateral IC and in control mice (Table 3 upper portion, t test, $P<0.05$-$0.001$). However, average latencies of IC neurons of control mice and the ipsilateral IC of experimental mice were not significantly different (t test, $P>0.1$). Bicuculline application either decreased, increased or did not change the latency of IC neurons. Figure 2 compares the latency of IC neurons of both groups of mice determined before and during bicuculline application. In control mice, bicuculline application produced a decrease in the latency of 14 (52%) neurons but did not change the latency of 13 (48%) neurons (Fig. 2A). In the ipsilateral IC of experimental mice, bicuculline application produced (1) a decrease in the latency of 19 (58%) neurons; (2) no change in the latency of 11 (33%) neurons and (3) a decrease in the latency of 3 (9%) neurons (Fig. 2B-i). In the contralateral IC of experimental mice, bicuculline application produced (1) a decrease in the latency of 19 (68%) neurons; (2) no change in the latency of 7 (25%) neurons and (3) a decrease in the latency of 2 (7%) neurons (Fig. 2B-c).

The upper half of Table 4 shows the average latencies of those IC neurons whose latencies were decreased by bicuculline application in both groups of mice. Bicuculline application significantly decreased the average latencies of these neurons (t test, $P<0.001$). Percent decrease in average latency was comparable (within 1%) for IC neurons in both groups of mice. In experimental mice, the average latencies of these neurons determined before and during bicuculline application were always significantly longer in the contralateral IC than in the ipsilateral IC (t test, $P<0.01$-$0.005$). Neurons in the IC of control mice and in the ipsilateral IC of experimental mice had comparable average latencies when determined both before and during bicuculline application (t test, $P>0.5$). In other words, bicuculline application produced a similar decrease in the latencies of IC neurons of control and the ipsilateral IC of experimental mice.

When measured before bicuculline application, neurons in the contralateral IC of experimental mice had

a significantly higher average MT than neurons in the ipsilateral IC and in control mice (Table 3 lower portion, t test, $P<0.05$). Average MTs of IC neurons of control mice and the ipsilateral IC of experimental mice were comparable (t test, $P>0.1$). Bicuculline application either decreased or did not affect the MTs of IC neurons. Figure 3 compares the MTs of IC neurons of both groups of mice determined before and during bicuculline application. In control mice, bicuculline application produced a decrease in the MTs of almost all (25, 93%) neurons (Fig. 3A). In the ipsilateral IC of experimental mice, bicuculline application produced a decrease in the MTs of 21 (64%) neurons but did not change the MTs of 12 (36%) neurons (Fig. B-i). In the contralateral IC of experimental mice, bicuculline application produced a decrease in the MTs of most (25, 89%) neurons but did not change the MTs of 3 (11%) neurons (Fig. 3B-c).

The lower half of Table 4 shows the average MTs of those IC neurons whose MTs were decreased by bicuculline application. Bicuculline application significantly decreased the average MT of these IC neurons in both groups of mice (t test, $P<0.001$). However, percent decrease in the average MT of IC neurons was two times greater for control mice than for experimental mice. In experimental mice, the average MTs determined both before and during bicuculline application were always significantly higher for neurons in the contralateral than in the ipsilateral IC (t test, $P<0.05$). The average MTs of neurons in control mice and in the ipsilateral IC of experimental mice obtained before and during bicuculline application were not significantly different (t test, $P>0.5$).

### *The Effect of Bicuculline Application on Threshold FTC*

Figure 4 shows the threshold FTCs of two representative IC neurons obtained before (predrug, a), during (bicuculline, b) and after (recovery, c) bicuculline application. Bicuculline application broadened the FTC of one neuron (Fig. 4Aa vs Ab) but did not affect the FTC of the other neuron (Fig. 4Ba vs Ba). FTCs of these two neurons measured before and after bicuculline application were quite comparable (Fig. 4 Aa vs Ac, Ba vs Bc).

The effect of bicuculline application on the sharpness of FTCs of these IC neurons was determined by comparing the $Q_{10}$ values of FTCs before and during bicuculline application. Before bicuculline application, neurons in the contralateral IC of experimental mice had

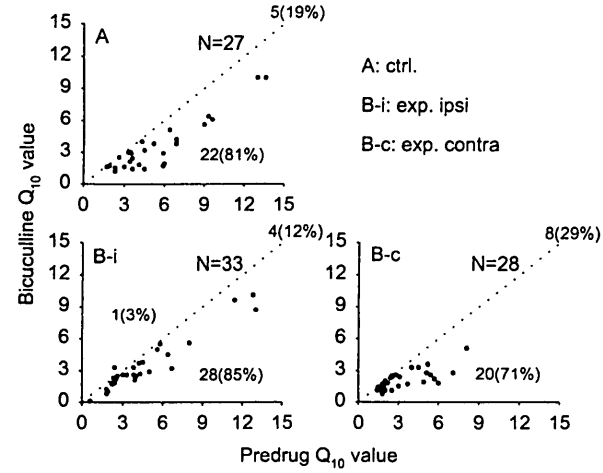


Fig. 5. Comparisons of $Q_{10}$ values of IC neurons obtained before (predrug) and during bicuculline application (See Fig. 2 for legends).

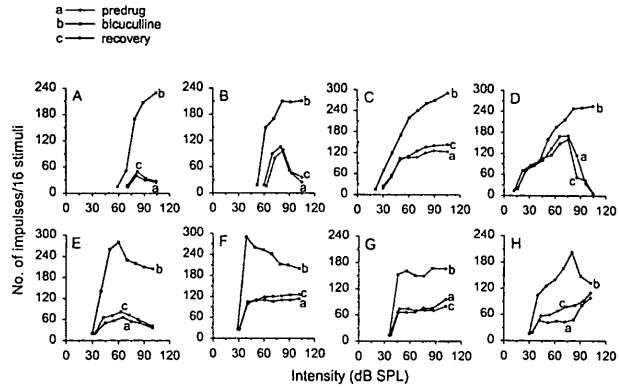


Fig. 6. Rate-intensity functions of eight representative IC neurons determined before (predrug, filled circles), during (bicuculline, filled squares) and after (recovery, unfilled circles) bicuculline application. Note that bicuculline application greatly increased the height of all rate-intensity functions. These eight neurons were recorded from the IC of control mice (A-C), the contralateral (E) and ipsilateral (D,F-H) ICs of experimental mice. The BF (kHz), MT (dB SPL), recording depth (μm) and latency (ms) of these eight neurons predrug were 12.9, 63, 1145, 13 (A); 12.2, 71, 650, 17 (B); 13.5, 30, 1000, 12 (C); 12.5, 16, 1220, 11 (D); 7.0, 35, 760, 15 (E); 9.2, 31, 1300, 11 (F); 9.1, 36, 760, 11 (G); 9.00, 32, 1000, 11 (H) (see text for details).

smaller average $Q_{10}$ value than neurons in the ipsilateral IC and in control mice although the difference was not significant (Table 5 upper portion, t test, $P>0.05$). In control mice, bicuculline application produced a decrease in the $Q_{10}$ values of 22 (81%) neurons but did not change the $Q_{10}$ values of 5 (19%) neurons (Fig. 5A). In the ipsilateral IC of experimental mice, bicuculline application produced (1) a decrease in the $Q_{10}$ values of 28 (85%) neurons; (2) no change in the $Q_{10}$ values of 4 (12%) neurons and (3) an increase in the $Q_{10}$ value of 1 (3%) neuron (Fig. 5B-i). In the contralateral IC of experimental mice, bicuculline application produced a decrease in the

$Q_{10}$ values of 20 (71%) neurons but did not change the $Q_{10}$ values of 8 (29%) neurons (Fig. 5B-c).

The upper half of Table 6 shows the average $Q_{10}$ values of those IC neurons whose $Q_{10}$ values were decreased by bicuculline application. Bicuculline application significantly decreased the average $Q_{10}$ value of these IC neurons in both groups of mice (t test, $P<0.001$). However, percent decrease in the average $Q_{10}$ value of IC neurons was largest for the contralateral IC, smallest for the ipsilateral IC of the experimental mice and intermediate for control mice. In experimental mice, the average $Q_{10}$ values for neurons in both colliculi determined before and during bicuculline application did not differ significantly (t test $P>0.5$).

### *The Effect of Bicuculline Application on Rate-intensity Functions*

Rate-intensity functions were obtained by plotting the number of impulses against the stimulus intensity. Fig. 6 shows rate-intensity functions of 8 representative IC neurons determined before (predrug, a), during (bicuculline, b) and after (recovery, c) bicuculline application. Bicuculline application increased the number of impulses of all IC neurons to varying degrees throughout the intensity range tested. The predrug and recovered rate-intensity functions of all but one (Fig. 6Ha vs Hc) neuron are literally congruent to each other.

All rate-intensity functions plotted before, during and after bicuculline application can be described as monotonic and nonmonotonic. The number of impulses of a monotonic neuron monotonically increased with stimulus intensity (Fig. 6Ab,Cb,Ha,c) or reached a plateau at high intensities (Fig. 6Bb,Ca,c,Fa,c,Ga,b,c). The number of impulses of a nonmonotonic neuron increased with stimulus intensity up to a maximum and then decreased more than 20% at still higher intensities (Fig. 6Aa,c,Ba,c,Da,c,Ea,c,Fb,Hb). In some extreme cases, the number of impulses decreased drastically at higher intensities such that rate-intensity functions had bell-shape (Fig. 6Ba,c, Da,c).

As shown in Table 7, most (73-78%) IC neurons from both groups of mice had more nonmonotonic rate-intensity functions before bicuculline application. Bicuculline application increased the number of monotonic neurons and decreased the number of nonmonotonic neurons in all ICs. To quantify the effect of bicuculline application on rate-intensity functions of these neurons, we compared their dynamic ranges calculated before and during bicuculline application. A

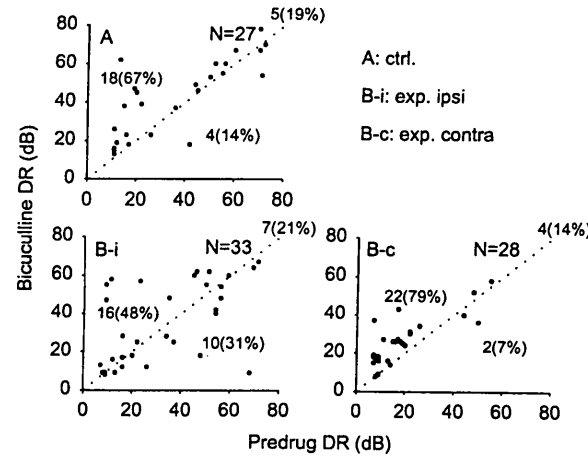


Fig. 7 Comparisons of dynamic ranges (DR) of IC neurons obtained before (predrug) and during bicuculline application (See Fig. 2 for legends).

dynamic range was defined as the intensity range corresponding to the number of impulses that was 10% below the maximum and 10% above the minimum of an rate-intensity function.

Before bicuculline application, neurons in the contralateral IC of experimental mice had significantly smaller average dynamic range than neurons in the ipsilateral IC and in control mice (Table 5 lower portion, t test, $P<0.05$-$0.0001$). Bicuculline application either increased, decreased or did not affect the dynamic range of IC neurons. Figure 7 compares the dynamic range of IC neurons of both groups of mice determined before and during bicuculline application. In control mice, bicuculline application produced (1) an increase in the dynamic range of 18 (67%) neurons; (2) no change in the dynamic range of 5 (19%) neurons and (3) a decrease in the dynamic range of 4 (14%) neurons (Fig. 7A). In the ipsilateral IC of experimental mice, bicuculline application produced (1) an increase in the dynamic range of 16 (48%) neurons; (2) no change in the dynamic range of 7 (21%) neurons and (3) a decrease in the dynamic range of 7 (21%) neurons (Fig. 7B-i). In the contralateral IC of experimental mice, bicuculline application produced (1) an increase in the dynamic range of 22 (79%) neurons; (2) no change in the dynamic range of 4 (14%) neurons; and (3) a decrease in the dynamic range of 2 (7%) neurons (Fig. 7B-c).

The lower half of Table 6 shows the average dynamic ranges of those IC neurons whose dynamic ranges were increased by bicuculline application. Bicuculline application significantly increased the average dynamic ranges of these IC neurons in both groups of mice (t test, $P<0.005$-$0.0001$). However, percent decrease in the average dynamic range of IC

neurons was largest for the ipsilateral IC, intermediate for the contralateral IC of the experimental mice and smallest for the control mice.

In experimental mice, the average dynamic ranges determined both before and during bicuculline application were always significantly smaller for neurons in the contralateral than in the ipsilateral IC (t test, $P<0.05$-$0.0001$). The average dynamic range of neurons in control mice and in the ipsilateral IC of experimental mice obtained before and during bicuculline application was not significantly different (t test, $P>0.5$).

## Discussion

### *Effects of Bicuculline Application on Response Properties of IC Neurons*

In this study, we examined the effect of bicuculline application on auditory response properties of IC neurons of mice with or without monaural middle ear destruction in early age. Sound stimulation was equal (symmetrical) at each ear of control mice. However, sound stimulation was asymmetrical so as to be weaker at the operated ear of experimental mice. We have observed that bicuculline application increased the number of impulses and discharge duration as well as changed discharge patterns of 43-67% of IC neurons in both groups of mice (Fig.1, Tables 1,2). Bicuculline application decreased the latency, lowered the MT, expanded FTC and increased the dynamic range of most IC neurons (Tables 4, 6). Similar observations have been reported for auditory neurons in many previous studies (6,11,13,17,18,24,25,35,36,51,52).

All these observations indicate that GABAergic inhibition contributes importantly to the dynamic aspect of auditory temporal processing. For example, it has been suggested that phasic discharge patterns are due to GABAergic inhibition that follows the neuron's excitatory responses to sound stimulation (4,5,20,32). The increase in number of impulses and decrease in response latency and MT upon bicuculline application has been attributed to the removal of GABAergic inhibition that precedes the neuron's excitatory response to the sound stimulation (5). Thus the frequency regions that expanded during bicuculline application were likely those under the control of GABAergic inhibitory neurons which would contribute to sharpening of the predrug excitatory FTC.

We found that bicuculline application did not affect the discharge patterns (e.g. Fig 1C-1, C-2,C-3, C4; Table 1 underlined), latencies, MTs and FTCs of some IC neurons (Figs 2,3, 4). It is conceivable that GABAergic inhibition either does not contribute to shaping the response properties of these neurons or they may simply inherited the response properties from subcortical auditory nuclei.

The fact that bicuculline application increased the number of impulses to varying degrees throughout the entire range of intensity (Fig. 6) suggests that GABAergic inhibition to each IC neuron was intensity-dependent. For example, we observed that bicuculline application could change a nomonotonic intensity-rate function into a monotonic one (e.g. Fig. 6 Da vs Db). This observation was most likely due to the fact that GABAergic inhibition was stronger at high than at low intensities such that release of GABAergic inhibition upon bicuculline application resulted in a greater increase in number of impulses at high than at low intensities. When GABAergic inhibition was stronger at low than at high intensities, bicuculline application typically did not change the type of rate-intensity function (e,g, Fig. 6 Ea vs Eb). However, the fact that more monotonic rate-intensity functions were obtained during bicuculline application (Table 7) suggests that most GABAergic inhibition was stronger at the high than at low intensities. The fact that the effect of bicuculline application on auditory response is intensity-dependent has been reported for bat IC neurons (6,37).

### *Difference in Response Properties between IC Neurons of Control and Experimental Mice*

We found that the ipsilateral IC of experimental mice had more tonic responders than the contralateral IC and control mice (Table 1). We also found that neurons in the IC contralateral to the operated ear had significantly longer latencies, higher MTs, and smaller dynamic ranges than neurons in the ipsilateral IC and control mice both before and during bicuculline application (Tables 3-6). The percent changes in latency, MT, $Q_{10}$ value and dynamic range during bicuculline application were also different between IC neurons of experimental and control mice (Tables 4, 6). What may be the possible mechanisms that contribute to these observed differences?

Previous studies have shown 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)(12,44). Because middle ear destruction

severely reduced the sound intensity reaching the inner ear (47,49,50), EI neurons ipsilateral to the operated ear receive stronger excitation from the unoperated ear relative to attenuated inhibition from the operated ear. In contrast, EI neurons contralateral to the operated ear receive weaker excitation from the operated ear relative to unattenuated inhibition from the unoperated ear. By the same token, EE or EO neurons ipsilateral to the operated ear receive stronger excitation from the unoperated ear than EE or EO neurons contralateral to the operated ear. Thus, the ipsilateral IC of experimental mice would receive stronger excitation from the unoperated ear and the contralateral IC would receive weaker excitation from the operated ear regardless of the aurality of IC neurons. These different degrees of excitation could be responsible for longer latencies, higher MTs and smaller dynamic ranges of neurons in the contralateral IC than in the ipsilateral IC of experimental mice and in control mice (Tables 2, 5). A reduced inhibition from the operated ear and an increased excitation from the unoperated ear might also conceivably contribute to two times more tonic responders in the ipsilateral IC than in the contralateral IC of experimental mice and control mice (Table 1).

### *Difference in the Effect of Bicuculline Application of Response Properties between IC Neurons of Control and Experimental Mice*

While bicuculline application produced comparable percent latency decreases in IC neurons of both groups of mice (8.5-9.4%, Table 4 top), it produced twice the percent MT decrease in IC neurons of control than experimental mice (19.6% vs 9.2%, Table 4 bottom). In contrast, the percent increase in the dynamic range of IC neurons during bicuculline application was 17-32% larger for experimental mice than for control mice (Table 6 bottom). In experimental mice, neurons in the contralateral IC had significantly longer response latency, higher MT and smaller dynamic range than neurons in the ipsilateral IC when determined both before and during bicuculline application (Tables 4,6). All these observations suggest that the unequal stimulation conditions created by monaural middle ear destruction may have affected the postnatal development of GABAergic receptors in experimental mice relative to control mice.

A recent study showed that unilateral cochlear ablation in adult gerbils produced a significant decrease in glutamic acid decarboxylase protein levels in the IC contralateral to the operated ear (31). This down-regulation of GABAergic systems increased the proportion of excited IC recording loci to sound stimulation of the intact, ipsilateral ear when compared with responses of IC neurons of the control animals with both cochleas intact (22,23,26,3134). As inhibitory and excitatory transmitter systems emerge at roughly at the same developmental age in the auditory nuclei (7,42,43), we have hypothesized in the Introduction that early monaural middle ear destruction may have produced disruption of normal development of the GABAergic transmitter systems in the IC of experimental mice. A disruption in the GABAergic transmitter systems by early monaural middle ear destruction may contribute to differential effects of bicuculline application on response properties of IC neurons in control and experimental mice (Table 2,4). Future immunocytochemical works are needed to confirm this possibility.

In summary, we have demonstrated that early monaural middle ear destruction affected only the percent distribution but not the type of discharge pattern, rate-intensity function and FTCs of IC neurons in the control and experimental mice. Neurons in the contralateral IC of experimental mice typically had longer latencies, higher minimum thresholds, broader FTCs and smaller dynamic ranges than neurons in the ipsilateral IC and in control mice. Bicuculline application produced differential effects in decreasing the latencies and MTs as well as broadening FTCs and dynamic ranges of IC neurons in these two groups of mice.

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