Bilateral Collicular Interaction: Modulation of Auditory Signal Processing in Amplitude Domain

Hui-Xian Mei1, Liang Cheng1, Jia Tang1, Zi-Ying Fu1, Xin Wang1, Philip H.-S. Jen2, Qi-Cai Chen1*

1 College of Life sciences and Hubei Key Lab of Genetic Regulation and Integrative Biology, Central China Normal University, Wuhan, Hubei, China, 2 Division of Biological Sciences, University of Missouri-Columbia, Columbia, Missouri, United States of America

Abstract

In the ascending auditory pathway, the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei, intrinsic projections within the IC, contralateral IC through the commissure of the IC and from the auditory cortex. All these connections make the IC a major center for subcortical temporal and spectral integration of auditory information. In this study, we examine bilateral collicular interaction in modulating amplitude-domain signal processing using electrophysiological recording, acoustic and focal electrical stimulation. Focal electrical stimulation of one (ipsilateral) IC produces widespread inhibition (61.6%) and focused facilitation (9.1%) of responses of neurons in the other (contralateral) IC, while 29.3% of the neurons were not affected. Bilateral collicular interaction produces a decrease in the response magnitude and an increase in the response latency of inhibited IC neurons but produces opposite effects on the response of facilitated IC neurons. These two groups of neurons are not separately located and are tonotopically organized within the IC. The modulation effect is most effective at low sound level and is dependent upon the interval between the acoustic and electric stimuli. The focal electrical stimulation of the ipsilateral IC compresses or expands the rate-level functions of contralateral IC neurons. The focal electrical stimulation also produces a shift in the minimum threshold and dynamic range of contralateral IC neurons for as long as 150 minutes. The degree of bilateral collicular interaction is dependent upon the difference in the best frequency between the electrically stimulated IC neurons and modulated IC neurons. These data suggest that bilateral collicular interaction mainly changes the ratio between excitation and inhibition during signal processing so as to sharpen the amplitude sensitivity of IC neurons. Bilateral interaction may be also involved in acoustic-experience-dependent plasticity in the IC. Three possible neural pathways underlying the bilateral collicular interaction are discussed.

Introduction

In sound reception, signal processing in higher centers of the auditory pathway is based on neural interactions from divergent and convergent projections through the interplay of excitation and inhibition [1]. For example, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many bilateral lower auditory nuclei as well as from the auditory cortex [2–11]. Neurons in one IC also receive projections within the IC and from the contralateral IC through the commissure of the IC [12–17]. For this reason, many studies have examined the interplay of excitation and inhibition in shaping the temporal processing and multiple-parametric selectivity in the IC [18–22]. Other studies have shown that the massive descending corticofugal system not only adjusts and improves ongoing collicular auditory signal processing in multiple-parametric domains but also reorganizes collicular auditory maps according to the acoustic experience [23–38].

Besides these numerous studies of the interplay of excitation and inhibition in afferent and efferent inputs to the IC, others have been devoted to examining the interaction between collicular neurons within the same IC and between the two ICs regarding auditory signal processing [15,39–42]. For example, when two neurons at different depths within the same IC are recorded under two-tone stimulation conditions, interaction between the two IC neurons produces inhibition (82%) and facilitation (18%) of the response of affected IC neurons. This colliculo-collicular interaction also sharpens the excitatory frequency tuning curves and decreases the rate-level function (RLF) of inhibited IC neurons through GABAergic inhibition [39,41,42]. Another study shows that focal electrical stimulation of collicular neurons evokes BF shifts of collicular neurons located near the stimulated ones and the collicular BF shifts depend on corticofugal feedback [37]. The collicular BF shift also depends on acetylcholine because it has been demonstrated that atropine (an antagonist of muscarinic acetylcholinergic receptors) applied to the IC blocks the development of collicular BF shifts [43]. Other studies examined the bilateral collicular interaction in signal processing by comparing the sound-evoked responses of one
IC neuron before and after hydraulic injection of kynurenic acid (antagonist of glutamic acid) into the corresponding region of the other IC \cite{15,40}. They indicate that the bilateral collicular interaction is mediated through the commissure of the IC to modulate the shape of the frequency response area, number of impulses and the shape of the RLFs of IC neurons. However, these studies did not determine if the degree of bilateral collicular interaction was related to the response parameters of neurons such as the best frequency, minimum threshold and latency of the neurons in the two ICs.

The main objective of this study is to examine the interaction of collicular neurons between the two ICs in amplitude-domain signal processing using electrophysiological recording, acoustic and focal electrical stimulation. Specifically, we study the effect of bilateral collicular interaction on amplitude sensitivity in relation to the tonotopy and plasticity in one IC during and after focal electrical stimulation of the other IC.

Methods

Animal Preparation and Surgery

A total of 21 (8 females, 13 males; body weight, b.w. 20–25 g) adult mice (\textit{Mus musculus}, Km) (2–3 months, supplied by the Center for Disease Control and Prevention of Hubei Province in China) was used for this study. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal University, Wuhan, Hubei, China. The surgical procedures for recording of sound-activated responses were basically the same as described in previous studies \cite{44,45}. Briefly, the flat head of a 2.0-cm nail was glued onto the exposed skull of each Nembutal anesthetized mouse (60–90 mg/kg b.w.) with acrylic glue and dental cement. Exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. After 1–2 hours of post-surgery, the anesthetized animal was tied to a metal plate inside a custom-made, double-wall, sound-proof room (temperature \(28^\circ\)–\(30^\circ\)C). The ceiling and inside walls of the room were covered with 2-cm polyurethane foam to reduce echoes. After fixing the head with a set screw and orienting the eye-nose line to 0\(^{\circ}\) in azimuth and 0\(^{\circ}\) in elevation of the frontal auditory space, small holes (diameter: 200–500 \(\mu\)m) were bored in the skull for the placement of the recording microelectrodes.

Table 1. The recording depth, BF, MT and latency of ICMdu neurons whose responses were inhibited or facilitated during ICES electrical stimulation.

| Inhibition Range | Depth (\(\mu\)m) | BF (kHz) | MT (dB SPL) | Latency (ms) |
|------------------|-----------------|----------|-------------|--------------|
| n = 61 mean \(\pm\) S.D. | 227–2003 | 5.5–27.6 | 15–87 | 8.5–23.5 |
| Facilitation Range | 390–1378 | 8.5–19.6 | 56–75 | 10.0–18.0 |
| n = 9 mean \(\pm\) S.D. | 1046.1–304.6 | 11.6–3.6 | 65–6.1 | 13.3–2.5 |
| t test, \(p\) | >0.05 | >0.05 | >0.05 | >0.05 |

Figure 1. Experimental arrangement and responses of ICES and ICMdu neurons under different stimulation conditions. A: A schematic drawing of a coronary section through the inferior colliculi (ICs) of mice (\textit{Mus musculus}, Km). The dashed lines delimit the central nuclei of the IC and its surrounding cortices. Filled grey circles indicate IC neurons which are bilaterally interconnected by the fibre projection (solid line) of the commissure of the IC. The drawing also shows the experimental arrangement for focal electrical stimulation and recording of the response of a neuron in one IC with a pair of custom-made tungsten electrodes (left) and recording of the response of a neuron in the other IC with a 2 M NaCl glass electrode (right). B: Peri-stimulus-time (PST) histograms showing the responses of two representative ICES neurons obtained before and after recovery from self focal electrical stimulation (a vs a’, b vs b’). C: PST histograms of inhibited (a vs a’) and facilitated (b vs b’) ICMdu neurons obtained before and during focal electrical stimulation of ICES neurons (abbreviated as ICES focal electrical stimulation). All PST histograms were obtained with a best frequency (BF) sounds delivered at 10 dB above the minimum threshold (MT). N: number of impulses in each PST histogram. Lat: response latency. Horizontal bar: acoustic stimulus. Arrows: focal electrical stimulus. The BF (kHz), MT (dB SPL) and recording depth (\(\mu\)m) of these four IC neurons were 11.3, 68, 740 (Ba); 14.1, 58, 859 (Bb); 15, 59, 1114 (Ca); 9.8, 71, 1378 (Cb). doi:10.1371/journal.pone.0041311.g001
in the skull above each IC for orthogonal insertion of custom-made tungsten electrodes (see below) and 2 M NaCl glass pipette electrode (tip diameter: <1 μm, impedance: 5–10 MΩ) for focal electrical stimulation and for recording sound-activated responses in the central nucleus of the IC. The depths of recorded IC neurons were read from the scale of two microdrives (David-Kopf, model 640, USA). A common indifferent electrode (silver wire) was placed at the nearby temporal muscles. Additional doses of anesthetics (one fourth of original) were administered during later phases of recording when the animal showed signs of discomfort as judged by increasing respiration and minor movement of limbs. In addition, a local anesthetic (Lidocaine) was applied to the open wound area to reduce any possible pain. When the animal was in good physiological condition, it was used up to 3 recording sessions on separate days, and each recording session typically lasted 2.6 hours to minimize the number of animals used for this study. Between recording sessions, the scalp was treated with antibiotic cream (Neosporin) to prevent inflammation and the skin was stitched back to the normal position before being put into the cage of animal room. The animal was then fed with food and water ad libitum until the next experimental session.

Stimulation and Isolation of Acoustically Activated Collicular (IC) Neurons

For acoustic stimulation, continuous sine waves from a function generator (GFG-8016G, Good Will Inst Co., Ltd, Bayan Lepas, Penang, Malaysia) were used. A fixed intensity of 80 dB was applied to the ear canal of the chinchilla, which was considered to be a comfortable level of sound. The sound stimulus was presented at a rate of 2 Hz for 30 s, followed by a 5 s silence. The BF and MT of IC neurons were measured using the procedures described above. The BF and MT of IC neurons were measured using the procedures described above. The BF and MT of IC neurons were measured using the procedures described above.

Figure 2. Correlation among different parameters of IC\textsubscript{Mdu} neurons. Scatter plots showing the distribution of the BF\textsubscript{s} of inhibited and facilitated IC\textsubscript{Mdu} neurons against recording depth (A), latency (B) and MT (C) as well as the BF difference against MT difference (D). Within each plot, the linear regression line and correlation coefficient are shown with a solid line and r. \( p \): significance level.

Figure 3. BF and MT differences of inhibited and facilitated IC\textsubscript{Mdu} neurons. Distribution histograms showing the BF (kHz) (A) and MT (dB) (B) differences of inhibited (A-1,B-1) and facilitated (A-2,B-2) IC\textsubscript{Mdu} neurons. Numbers in the right abscissa indicate that IC\textsubscript{Mdu} neurons had larger BF and MT than IC\textsubscript{ES} neurons. The opposite is shown in the left abscissa. The mean and standard deviation of each group of neurons (n) are shown. \( p \): significance level of \( t \) test.

A-3: the average BF and MT differences of inhibited and facilitated IC\textsubscript{Mdu} neurons. The number of neurons and half a standard deviation are shown atop of each bar.
Penang, Malaysia) were formed into 40 ms pure tones (5 ms rise-decay times) with a custom-made tone burst generator (electronic switch) driven by a stimulator (Model SEN-7203, Nihon Kohden Co, Shinjuku, Tokyo, Japan) and delivered at 2 pulses per second. The tone pulses were then amplified (custom-made amplifier) after passing a decade attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan) before they were fed into a small loudspeaker (AKG model CK 50, 1.5 cm in diameter, 1.2 g, frequency response 1–100 kHz). The loudspeaker was calibrated with a 1/4-inch microphone (4939, B&K, Denmark) placed at the mouse’s ear using a measuring amplifier (2610, B&K, Denmark). The output of the loudspeaker was expressed in decibel sound pressure level (dB SPL) in reference to 20 μPa root mean square. A frequency response curve of the loudspeaker was plotted to determine the maximal available sound amplitude at each frequency. The maximal stimulus level ranged from 95 to 120 dB SPL between 10 and 80 kHz but dropped off sharply to 80 dB SPL at 100 kHz thereafter.

Two insulated tungsten electrodes (FHC Inc, Bodowin, ME, USA) were glued together (tip: <10 μm, inter-tip distance: ≤100 μm) to form a pair of tungsten electrodes. These electrodes were used for recording sound-activated responses of IC neurons and for focal electrical stimulation in the IC recording site (4 ms train of four monophasic pulses of 0.1 ms with 0.9 pulse-gap at 2 train/s, 5–50 μA) using stimulator (Model SEN-7203, Nihon Kohden CO, Tokyo, Japan) and stimulus isolation unit (Model Nihon Kohden CO, Tokyo, Japan/Fig. 1A, left).

During experiment, a 40 ms sound was delivered (at 2 pulses/s) from the loudspeaker placed 30 cm away from the animal and 60° contralateral to the recording site in order to maximally excite the recorded IC neuron [44,45]. When an IC neuron was isolated (the first IC neuron, abbreviated as the ICES neuron) with a pair of custom-made tungsten electrodes, its best frequency (BF) and minimum threshold (MT) were audio-visually measured by systematically changing the frequency and level of sound pulses. The sound frequency that elicited the neurons’ response at the lowest amplitude was defined as the BF. The threshold at the BF

Figure 4. Variation of modulation of IC_{du} neurons with inter-stimulus interval and BF and MT differences. Variation in the number of impulses (solid squares refer to left ordinates) and the response latency (unfilled square refer to right ordinates, S1 in C) of two IC_{du} neurons during ICES focal electrical stimulation at each inter-stimulus interval (ISI in ms). At each ISI, there was an inhibitory (A-1) or facilitatory (B-1) latency (S2 in C). The smallest number of impulses (solid arrow) and the longest acoustic response latency (unfilled arrow) were always obtained at the best inhibitory latency for the inhibited IC_{du} neuron but the opposite were obtained at the best facilitatory latency for the facilitated IC_{du} neuron. The BF (kHz), MT (dB SPL) and recording depth (μm) of these two IC_{du} neurons were 9.8, 64, 634 (Aa); 9.3, 59, 390 (Ab). A-2,A-3,B-2,B-3: The scatter plots showing the best inhibitory latency (solid circles, n = 61) and the best facilitatory latency (unfilled circles, n = 9) of IC_{du} neurons in relation to BF and MT differences between ICES and IC_{du} neurons. A linear regression line and correlation coefficient are shown with a solid line and r, p significance level. C: A sketch showing the PST histogram of a hypothetical IC_{du} neuron in response to acoustic stimulus (AS) combined with ICES electrical stimulation (ES). S1: the acoustically activated response latency; S2: the inhibitory or facilitatory latency expressed as the time interval between the onset of ES and auditory response. D: the mean best affected latency of 61 inhibited (I) and 9 facilitated (F) IC_{du} neurons. The number of neurons and half of a standard deviation are shown atop of each bar.

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was defined as the MT. At the MT, the neuron, on average, responded with 50% probability to BF pulses.

Acoustically activated responses of an IC neuron in the other IC (the second IC neuron, abbreviated as the IC Mdu neuron) was then isolated with a 2 M NaCl glass electrode after moving the loudspeaker 60° contralateral to the isolated IC Mdu neuron (Fig. 1A, right). After determining its BF and MT, its response to BF sound pulses delivered at 10 dB above the MT was recorded as the control response. The neuron’s response was then monitored again during focal electrical stimulation in the ICES neuron (hereafter abbreviated as ICES focal electrical stimulation) through the custom-made tungsten electrodes. Electrical stimulus was synchronized with the acoustic stimulus by a synchrony trigger signal (2 pulses/s) from the stimulator (Model SEN-7203, Nihon Kohden Co, Shinjuku, Tokyo, Japan) which triggered the custom-made tone burst generator and an electric stimulator such that the interval between the two stimuli could be adjusted at random. At first, the electrical stimulation was delivered at 2 trains/s between 5 and 50 μA and at a randomly chosen inter-stimulus interval (ISI). The current level was gradually increased in order to find an IC Mdu neuron affected by the ICES electrical stimulation and to observe the effect on response of the IC Mdu neuron under different current level. Then, the electrical stimulation current was fixed at moderate level (25 μA) and the ISI was adjusted systematically to determine the optimal ISI that produced maximal effect. If the percent change in number of impulses of the IC Mdu neuron induced by the ICES electrical stimulation didn’t reach 30%, the IC Mdu neuron was abandoned. Otherwise it was regarded as a modulated IC Mdu neuron. At the optimal ISI, the response latency and RLF of the modulated IC Mdu neuron were then measured before and during ICES focal electrical stimulation. The response latency was defined as the interval between the onset of the acoustic stimulus and the neuronal response. A RLF was measured with a neuron’s number of impulses obtained with a BF sound delivered at MT and at 10 dB increments above the MT.

As in previous studies [23–25,31,38], the modulation of the response of IC Mdu neurons disappeared upon the cessation of ICES focal electrical stimulation when delivered at 2 trains/s at 25 μA. Therefore, to study the plasticity of the responses of IC Mdu neurons, ICES focal electrical stimulation was delivered at the optimal ISI and 10 trains/s for 30 minutes synchronized with the intervals.

![Figure 5. Modulation of the rate-level function of IC Mdu neurons after 30 minute ICES focal electrical stimulation.](image1)

**Figure 5.** Modulation of the rate-level function of IC Mdu neurons after 30 minute ICES focal electrical stimulation. The rate-level function (RLF) of an inhibited (A) and a facilitated (B) IC Mdu neuron measured before (unfilled circles) and at different times (at 0 min, a; 30 min, b; 60 min, c; 90 min, d; 120 min, e, filled circles) after 30 minute ICES focal electrical stimulation. The dynamic range (DR) of the control RLF (unfilled circles) and modulated RLF (filled circles) are shown. C,D: The time course of DR shift of these two IC Mdu neurons after 30 minute ICES focal electrical stimulation (indicated with short horizontal bar). Downward curve(C) indicates DR is decreased, while upward curve (D) indicates the opposite. The BF (kHz), MT (dB SPL) and recording depth (μm) of these two IC Mdu neurons were 16.9, 68, 1205 for A and 12.2, 53, 954 for B. doi:10.1371/journal.pone.0041311.g005

![Figure 6. Correlation among DR and MT shifts and the MT of IC Mdu neurons.](image2)

**Figure 6.** Correlation among DR and MT shifts and the MT of IC Mdu neurons. Scatter plots of the MT shift against DR shift and MT of IC Mdu neurons. N: number of IC Mdu neurons. The linear regression line and correlation coefficient are shown with a solid line and r. p: significance level. doi:10.1371/journal.pone.0041311.g006
The onset of acoustic stimulus (the BF of IC 

neuron delivered at 10 dB above its MT). The discharge pattern and the RLF of the IC 

neuron were then progressively monitored at 0, 30, 60, 90, 120, 150 minutes after 30 minute ICES focal electrical stimulation.

Data Collection and Analysis

An IC neuron’s response under different stimulation conditions was amplified and band-pass filtered (ISO-DAM, WPI, USA) before being sent to an oscilloscope (TDS210, Tek, USA) and an audio monitor (Grass AM9, USA). The neuron’s response was also sent to a computer (Kaitian 4600, Lenovo, China) for acquisition of peri-stimulus-time (PST) histograms (bin width: 250 ms, sampling period: 150 ms) to 32 sound presentations. The PST histogram showed the neuron’s temporal discharge pattern to sound stimulus. The total number of impulses in each histogram was used to quantify the neuron’s response under each stimulus condition.

The modulation of response of each IC 

neuron by ICES focal electrical stimulation was studied by calculating the change in the control number of impulses and dynamic range (DR) of the RLF of the IC 

neuron obtained before, during or after ICES focal electrical stimulation. A DR of a RLF is the range of the stimulus level defined by a neuron’s response magnitude at 10% above the minimum and below the maximum. All the BF and MT differences between ICES and IC 

neuron and the shifts in different parameters of IC 

neurons during or after relative to before electrical stimulation are calculated in absolute values. All data obtained under different stimulation conditions were processed and plotted using Sigmaplot 2000. They were then quantitatively examined and statistically compared using SPSS 13.0 (one-way ANOVA, p < 0.05) and Student’s t test at p < 0.05).

Results

Inhibition and Facilitation of Responses of IC 

neurons during ICES Focal Electrical Stimulation

Focal electrical stimulation in the IC neurons did not appear to affect their acoustically activated responses which recovered to the control level right after the electrical stimulation (Fig. 1Ba vs Ba’; Bb vs Bb’). Among 99 IC 

neurons isolated, the responses of 29 neurons were not modulated during ICES focal electrical stimulation. In the remaining 70 IC 

neurons, ICES focal electrical stimulation produced a decrease in the number of impulses (30–75%, average: 40.1 ± 11%) and an increase in the response latency (0.1–3 ms, average: 1 ± 0.9 ms) of 61 (87%) inhibited IC 

neurons (Fig. 1Ca vs Ca’). Conversely, ICES focal electrical stimulation produced an increase in the number of impulses (34.8–91%, average: 60.2 ± 21.4%) and a decrease in the response latency (0.5–2.5 ms, average: 1.1 ± 0.7 ms) of 9 (13%) facilitated IC 

neurons (Fig. 1Cb vs Cb’).

As shown in Table 1, these two groups of IC 

neurons did not differ in the recording depth, BF, MT and latency indicating that they are not separately located within the IC. They were tonotopically organized within the IC such that their BF progressively increased with the recording depth (Fig. 2A). However, no correlation was found between the latency and BF or between the BF and MT of these IC 

neurons or between the BF and MT differences of ICES and IC 

neurons (Fig. 2B,C,D. p > 0.05). These findings suggest that IC 

neurons
in each iso-frequency lamina might have similar BFs but quite
different MTs. These findings are in agreement with those
reported in previous studies [46–49].

Figure 3 shows the distribution histograms of BF and MT
differences of these inhibited and facilitated IC_{Mdu} neurons. It is
clear that both inhibited and facilitated IC_{Mdu} neurons had higher
or lower BF and MT than corresponding IC_{ES} neurons such that
their BF and MT differences were bilaterally distributed. Although
these bilateral BF and MT differences did not differ significantly (t
test, p > 0.05), inhibited IC_{Mdu} neurons had wider distribution of
BF and MT differences than facilitated IC_{Mdu} neurons (Fig. 3A-
B-1, B-2 vs A-2, B-2). The BF and MT differences of inhibited IC_{Mdu}
neurons were mostly less than 5 kHz (47/61, 77%) and 20 dB (49/
61, 80%) while they were all less than 2 kHz and 15 dB for
facilitated IC_{Mdu} neurons. As such, the former had larger average
BF and MT differences than the latter had (Fig. 3A-3, 2.9±2.5 vs
0.9±0.3 kHz; Fig. 3B-3, 13.5±10.8 vs 6.4±4.1 dB).

The degree of modulation of IC_{Mdu} neurons produced by IC_{ES}
focal electrical stimulation varied with the interval between
acoustic and electrical stimuli (ISI). As the ISI was systematically
varied such that the electrical stimulus first appeared before,
and then after the acoustic stimulus, the number of
impulses of the inhibited IC_{Mdu} neuron decreased from a large
number to a minimum at the optimal ISI and increased thereafter
with further variation in the ISI (Fig. 4A-1, left ordinate). In
contrast, the neuron’s response latency increased from a short
latency to the longest one at the optimal ISI before decreasing to
a short one again with further variation in the ISI (Fig. 4A-1, right
ordinate). The opposite effects were observed for the facilitated
IC_{Mdu} neuron. The neuron’s number of impulses increased from
a minimum to the maximum at the optimal ISI before decreasing
to another minimum with further variation in the ISI (Fig. 4B-1,
left ordinate). Conversely, the neuron’s response latency decreased
from a long latency to the shortest one at the optimal ISI and it
then increased to another long one with further variation in the ISI
(Fig. 4B-1, right ordinate). As in the previous study [50], we
defined the inhibitory latency that produced the longest response
latency at the optimal ISI as the best inhibitory latency (arrow in
Fig. 4A-1, abscissa). We also defined the facilitatory latency that
produced the shortest response latency at the optimal ISI as the
best facilitatory latency (arrow in Fig. 4B-1, abscissa). In this study,
the average optimal ISI was 2.1±1.5 ms (range: 0–7 ms) for 61
inhibited IC_{Mdu} neurons and 2.6±2.2 ms (range: 0–8 ms) for 9
facilitated IC_{Mdu} neurons.

Figure 8. Modulation of IC_{Mdu} neurons during IC_{ES} focal electrical stimulation. Focal electrical stimulation of one IC_{ES} neuron produced
inhibition of two IC_{Mdu} neurons and facilitation of one IC_{Mdu} neuron (A, B). Focal electrical stimulation of another IC_{ES} neuron produced inhibition of
all three IC_{Mdu} neurons (C, D). The PST histogram, number of impulses (N), recording depth and BF of all IC_{ES} and IC_{Mdu} neurons are shown. %: percent
inhibition or facilitation of IC_{Mdu} neurons. Arrow: IC_{ES} focal electrical stimulation. Bottom: a cartoon showing the divergent pattern of connections from
an injection site of one IC through the commissure of the IC to different frequency laminae of the other IC (adapted from [51]).
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Linear regression analyses of the scatters plots of the best inhibitory latency of IC Mdu neurons against BF and MT differences showed a significant correlation of the best inhibitory latency with the BF difference but not with the MT difference (Fig. 4A-2 vs A-3). However, similar analyses were not performed for the 9 facilitated IC Mdu neurons because of the small sample size and narrow range of BF and MT differences (Fig. 4B-2,B-3). The average best inhibitory latency of 61 inhibited IC Mdu neurons was 16.6 ± 5 ms (range: 9–29 ms) which was longer than the average best facilitatory latency of 9 facilitated IC Mdu neurons (13.2 ± 1.9 ms, range: 11.5–17.5 ms) (Fig. 4D).

The Time Course of Modulation of the RLF of IC Mdu Neurons after 30 Minute ICES Focal Electrical Stimulation

To determine the time course of modulation of the RLF of IC Mdu neurons, we measured their RLFs at different time frames after 30 minutes of ICES focal electrical stimulation. As shown in Fig. 5, the representative inhibited IC Mdu neuron had a monotonic RLF in which the neuron’s number of impulses progressively increased with sound level. The neuron’s RLF was decreased to varying degree with sound level resulting in a decreased DR after 30-minute ICES focal electrical stimulation (Fig. 5A unfilled vs filled circles, DR decreased from 26.5 dB to 17 dB). The decreased RLF and DR slowly returned to the control level (measured before ICES focal electrical stimulation) over a period of more than 120 minutes. The largest DR shift (decrease) occurred right after the 30 minute ICES focal electrical stimulation (Fig. 5Ab,C).

Opposite to these observations, the representative facilitated IC Mdu neuron had a non-monotonic RLF in which the neuron’s number of impulses progressively increased with sound level up to a maximum but sharply decreased thereafter at still higher sound level (Fig. 5B). The neuron’s RLF was elevated to varying degree with sound level resulting in an increased DR after 30 minute ICES focal electrical stimulation (Fig. 5B unfilled vs filled circles, DR increased from 32.7 dB to 41.2 dB). The elevated RLF and increased DR slowly returned to the control level over a period of 90 minutes. The largest DR shift (increase) occurred right after the 30 minute ICES focal electrical stimulation (Fig. 5Ab,C).

Among 31 IC Mdu neurons studied, the recovery time of DR shift produced by 30 minute ICES focal electrical stimulation was within 30 minutes in 5 neurons, 60 minutes in 9 neurons, 90 minutes in 9 neurons, 120 minutes in 6 neurons and 150 minutes in 2 neurons.

The DR and MT shifts of IC Mdu neurons produced by ICES focal electrical stimulation did not bear any relationship with the DR and MT of electrically stimulated ICES neurons. However, linear regression analyses of the scatter plots of the DR and MT shifts and the MT of IC Mdu neurons revealed that the DR and MT
for the 9 facilitated ICMdu neurons due to small sample size.

The percent inhibition for the two inhibited ICMdu neurons was larger for the neuron with a smaller BF difference of 0.8 kHz. The percent inhibition and facilitation of ICMdu neurons reduced sharply with increasing sound level (Fig. 7Ab, Bb). On average, the inhibition and facilitation of ICMdu neurons during IES focal electrical stimulation greatly reduced with sound level within 20–30 dB above the MT before reaching a plateau value at still higher sound levels (Fig. 7Ac, Bc).

**Discussion**

Modulation of ICMdu Neurons by IES Focal Electrical Stimulation

In this study, we used an electrical stimulus of 25 μA to activate IES neurons, similar to those used in previous studies [23,24,31,50]. This focal electrical stimulation can effectively activate IES neurons without changing their auditory response properties (Fig. 1B vs a1; B vs b1). This IES focal electrical stimulation respectively weakens and strengthens the effectiveness of a sound stimulus through inhibition and excitation of modulated ICMdu neurons. As a result, the number of impulses and latency of inhibited ICMdu and facilitated ICMdu neurons changed in opposite ways and varied with the ISI (Figs. 1C, 4A-1, B-1). The fact that inhibited ICMdu neurons had larger BF and MT differences than facilitated ICMdu neurons suggests that bilateral collicular interaction is mediated through wide spread inhibition and focused facilitation (Fig. 3A-3, B-3).

The degree of modulation of ICMdu neurons produced by IES focal electrical stimulation was the greatest at MT level but decreased progressively with sound level (Fig. 7). Conceivably, this observation is due to the fact that bilateral collicular interaction produces a constant amount of inhibitory or facilitatory modulation of ICMdu neurons at all sound levels and the effectiveness of modulation progressively decreases when the excitation of ICMdu neurons increases with sound level. This observation is consistent with a previous study that shows that bilateral collicular interaction can mediate both excitatory and inhibitory effects via the commissure of the IC and the greatest modulating effects occurring at near-threshold levels [40]. A similar observation has also been reported in previous studies of corticofugal modulation and forward masking modulation of IC neurons [22,23,39,41,43,50].

ICES focal electrical stimulation compressed the RLF, decreased the DR and increased the MT of inhibited ICMdu neurons but produced opposite effects on facilitated ICMdu neurons, the induced shift in MT and DR is significantly correlated (Figs. 5, 6A). Conceivably, the role of bilateral collicular interaction is to sharpen the amplitude sensitivity of inhibited ICMdu neurons through wide spread inhibition and to enhance responsiveness of facilitated ICMdu neurons to tuned sound stimulus through focused facilitation. Since 30 minute IES focal electrical stimulation also produced a long term shift in DR and MT of ICMdu neuron, the bilateral collicular interaction may be also involved in acoustic-experience-dependent plasticity in the IC.

We observed that IES focal electrical stimulation produced greater MT shifts for ICMdu neurons with lower than with higher MT (Fig. 6B). This is perhaps due to the fact that ICMdu neurons with higher MT would require stronger sound for excitation and the modulation effect of IES focal electrical stimulation is most effective at low than at high sound level (Fig. 7).

Modulation of ICMdu Neurons is BF-difference Dependent

Previous studies indicate that the two ICs have tonotopically appropriate reciprocal connections with each other [12,14,17,51]. This well organized tonotopic organization of both ICs suggests
that IC\textsubscript{Mdu} neurons with small BF differences would receive stronger collicular interaction influences than IC\textsubscript{Mdu} neurons with large BF differences. In other words, modulation effect produced by IC\textsubscript{ES} focal electrical stimulation attenuates with distance along the tonotopic axis of the IC. This is supported by our findings that the inhibited IC\textsubscript{Mdu} neurons with smaller BF differences have shorter best inhibitory latency, larger inhibition and shift in DR and latency than inhibited IC\textsubscript{Mdu} neurons with larger BF differences had (Figs. 4A-2, 9A-1, B-1,D-1).

We observed that the MT shift produced by IC\textsubscript{ES} focal electrical stimulation is significantly correlated with both the MT of IC\textsubscript{Mdu} neurons and the MT difference (Figs. 6B, 9C-2). Also, the BF shift produced by IC\textsubscript{ES} focal electrical stimulation not only is significantly correlated with the BF difference but also with the DR and MT shift (Fig. 10A,C,D). These observations are quite different from a previous study in bat which shows that BF, MT and DR shift produced by corticofugal modulation is only significantly correlated with BF, DR and MT differences between collicular and cortical neurons, respectively [25,38]. These differences suggest that corticofugal and bilateral collicular modulation of amplitude signal processing in the IC is comple-ment but not entirely comparable.

Possible Neural Pathways Underlying the Bilateral Collicular Interaction

What are the possible neural pathways underlying bilateral collicular interaction? As described earlier, each IC receives multiple inputs from many bilateral lower auditory nuclei, the auditory cortex, intrinsic projections within the IC and from the contralateral IC through the commissure of the IC [2-17]. Therefore, there are at least three possible pathways that can mediate the bilateral interactions observed in the present study. First, IC\textsubscript{ES} focal electrical stimulation produces bilateral collicular interaction through the commissure of the IC. Second, IC\textsubscript{ES} focal electrical stimulation activates the ascending pathways to directly or indirectly excite the ipsilateral auditory cortex which subsequently modulates the response of contralateral IC directly or through the contralateral auditory cortex by way of the corpus callosum. Third, IC\textsubscript{ES} focal electrical stimulation activates the descending pathways to excite neurons in the lower auditory nuclei which subsequently modulate the response of contralateral IC through multiple ascending neural pathways.

In the present study, we showed that modulation of IC\textsubscript{Mdu} neurons by IC\textsubscript{ES} focal electrical stimulation is closely correlated

with BF difference (Figs. 8, 9A-1,C-1,D-1, 10A). These findings are nicely corroborated by a recent anatomical study of the topographical organization of the commissural connections between two ICs [31]. This study reveals that commissural neurons in the central nucleus of IC send a divergent projection to the equivalent frequency-band laminae in the corresponding central nucleus of IC and the density of this projection is greatest between corresponding points; consistent with a point-to-point emphasis in the wiring pattern (cartoon in Fig. 9). Conceivably, this divergent projection from one IC to the frequency-band laminae of the contralateral IC may be the anatomical basis underlying the BF difference-dependent modulation of IC\textsubscript{Mdu} neurons during IC\textsubscript{ES} focal electrical stimulation. Because facilitated IC\textsubscript{Mdu} neurons have smaller BF differences than inhibited IC\textsubscript{Mdu} neurons have [Fig. 3A-3], the former may be mediated by the more focused point-to-point connections between corresponding frequency laminae in two ICs and the latter may be mediated by the divergent connections between non-corresponding frequency laminae in two ICs. If this is true, the facilitated IC\textsubscript{Mdu} neurons would have a shorter best affected latency than inhibited IC\textsubscript{Mdu} neurons had (Fig. 4D).

Previous studies indicate that focal cortical electrical stimulation not only evoke cortical, thalamic and collicular BF shifts but also evoke subcortical BF shifts [36,52]. In addition, it has been shown that the collicular BF shift evoked by electrical stimulation of the neighboring collicular neuron is mediated mainly through ipsilateral corticofugal feedback [37]. Therefore, future studies are necessary to determine if bilateral collicular interaction might also be mediated through the corticofugal feedback loop and/or subcortical pathways. These studies may involve the inactivation of the ipsilateral auditory cortex with Lidocaine and/or by ablation of the commissure of the IC during IC\textsubscript{ES} focal electrical stimulation.

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Author Contributions

Conceived and designed the experiments: CQ-C. Performed the experiments: H-XM LC JT Z-YF XW. Analyzed the data: MH-X LC JT Z-YF PHSJ CQ-C. Contributed reagents/materials/analysis tools: QC-C. Wrote the paper: H-XM CQ-C PHSJ.

References

1. Saga N (1997) Parallel-hierarchical processing of complex sounds for specialized auditory function. In: Crocker MJ, editor. Encyclopedia of Acoustics. New York: Wiley: 1409–1418.
2. Adams JC (1979) Ascending projections to the inferior colliculus. J Comp Neurol 183: 519–538.
3. Bajo VM, Moore DR (2005) Descending projections from the auditory cortex to the inferior colliculus in the rat: a quantitative study using anterograde and retrograde transport. J Comp Neurol 486: 101–116.
4. Cant NB, Benson CG (2006) Organization of the inferior colliculus of the gerbil (Meriones unguiculatus): differences in distribution of projections from the cochlear nuclei and the superior olivary complex. J Comp Neurol 495: 511–529.
5. Cessford JH, Fremouw T, Govey E (2002) The inferior colliculus: a hub for the central auditory system. In: Oertel D, Fay RR, Popper AN, editors. Integrative Functions of the Mammalian Auditory Pathway. New York: Springer. 230–318.
6. Herbert H, Aschoff A, Ostwald J (1991) Topography of projections from the auditory cortex to the inferior colliculus in the rat. J Comp Neurol 304: 103–122.
7. Malmierca MS, Saint Marie RL, Merchán MA, Oliver DL (2005) Laminar point-to-point connections between corresponding frequency-band laminae in two ICs. If this is true, the facilitated IC\textsubscript{Mdu} neurons would have a shorter best affected latency than inhibited IC\textsubscript{Mdu} neurons had (Fig. 4D).
8. Saldeta E, Feliciano M, Mugnaini E (1996) Distribution of descending projections from primary auditory neocortex to inferior colliculus mimics the topography of intracortical projections. J Comp Neurol 371: 13–40.
9. Shneiderman A, Oliver DL (1989) EM autoradiographic study of the projections from the dorsal nucleus of the lateral lemniscus: a possible source of inhibitory inputs to the inferior colliculus. J Comp Neurol 268: 47–47.
10. Winer JA, Larue DT, Dieder JH, Hefi BJ (1998) Auditory cortical projections to the cat inferior colliculus. J Comp Neurol 400: 147–174.
11. Winer JA (2006) Decoding the auditory corticofugal systems. Hear Res 212: 1–8.
12. Aitkin LM, Phillips SC (1984) The interconnections of the inferior colliculus through their commissure. J Comp Neurol 221: 210–216.
13. Hernández O, Rees A, Malmierca MS (2006) A GABAergic component in the commissure of the inferior colliculus in rat. Neuroreport 17: 1611–1614.
14. Malmierca MS, Rees A, Le Beau FE, Bjaalie JG (1995) Laminar organization of frequency-defined focal axons within and between the inferior colliculi of the guinea pig. J Comp Neurol 357: 124–144.
15. Malmierca MS, Hernández O, Falcón A, Lopez-Poveda EA, Merchán M, et al. (2003) The commissure of the inferior colliculus shapes frequency response areas in rat: an in vivo study using reversible blockade with microiontophoresis of kynurenic acid. Exp Brain Res 153: 522–529.
16. Moore DR, Kotak VC, Sanes DH (1998) Commisural and lemniscal synaptic input to the gerbil inferior colliculus. J Neurophysiol 80: 2229–2236.
