● RESEARCH ARTICLE

Responses from two firing patterns in inferior colliculus neurons to stimulation of the lateral lemniscus dorsal nucleus

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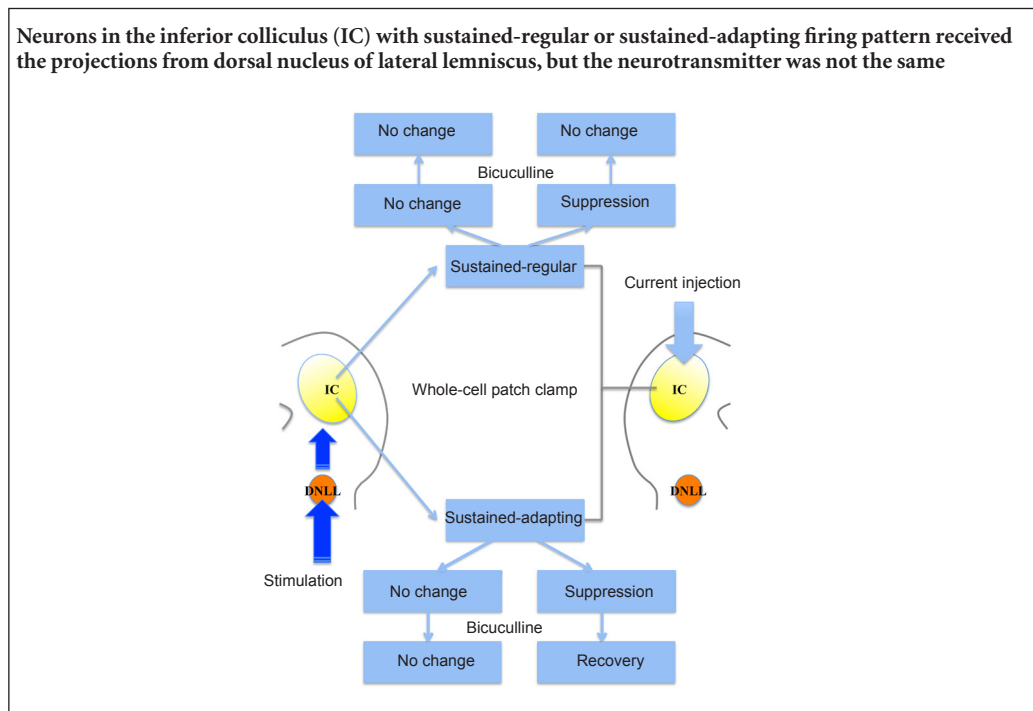
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Graphical Abstract

Neurons in the inferior colliculus (IC) with sustained-regular or sustained-adapting firing pattern received the projections from dorsal nucleus of lateral lemniscus, but the neurotransmitter was not the same

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Abstract

The γ-aminobutyric acid neurons (GABAergic neurons) in the inferior colliculus are classified into various patterns based on their intrinsic electrical properties to a constant current injection. Although this classification is associated with physiological function, the exact role for neurons with various firing patterns in acoustic processing remains poorly understood. In the present study, we analyzed characteristics of inferior colliculus neurons *in vitro*, and recorded responses to stimulation of the dorsal nucleus of the lateral lemniscus using the whole-cell patch clamp technique. Seven inferior colliculus neurons were tested and were classified into two firing patterns: sustained-regular (*n* = 4) and sustained-adapting firing patterns (*n* = 3). The majority of inferior colliculus neurons exhibited slight changes in response to stimulation and bicuculline. The responses of one neuron with a sustained-adapting firing pattern were suppressed after stimulation, but recovered to normal levels following application of the γ-aminobutyric acid receptor antagonist. One neuron with a sustained-regular pattern showed suppressed stimulation responses, which were not affected by bicuculline. Results suggest that GABAergic neurons in the inferior colliculus exhibit sustained-regular or sustained-adapting firing patterns. Additionally, GABAergic projections from the dorsal nucleus of the lateral lemniscus to the inferior colliculus are associated with sound localization. The different neuronal responses of various firing patterns suggest a role in sound localization. A better understanding of these mechanisms and functions will provide better clinical treatment paradigms for hearing deficiencies.

Introduction

The inferior colliculus (IC) is a vital relay station in the central auditory pathway; it integrates acoustic information from lower brainstem nuclei, such as the dorsal cochlear nucleus complex, the superior olivary complex, and the lateral lemniscal nuclei (Adams, 1979; Malmierca et al., 2005; Oliver, 2005). These projections provide either excitatory or inhibitory innervation, or both of them, and neurons in the IC are responsible for interactions between excitatory and inhibitory inputs (Davis, 2002; Malmierca et al., 2005).

Previous studies have shown that inhibition occupies a dominant role in the processing of acoustic information (Kelly and Caspary, 2005; Nataraj and Wenstrup, 2005; Fuzessery et al., 2006; Pollak et al., 2011; Geis and Borst, 2013). In the IC, γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter (González-Hernández et al., 1996; Chen et al., 1999; Merchán et al., 2005); GABA affects auditory processing in many ways, including duration tuning, selectivity of sweep direction of frequency modulation, and coding of interaural time or intensity differences (Yin et al., 2008; Pollak et al., 2011). Within the IC, GABA is mainly originated from the bilateral dorsal nucleus of the lateral lemniscus (DNLL), especially in the contralateral side (51% in a study) (Adams, 1979; Shneiderman et al., 1993; González-Hernández et al., 1996; Zhang et al., 1998; Burger and Pollak, 2001; Oliver, 2005; Winter and Schreiner, 2005; Pollak et al., 2011). The intrinsic projections in the IC subdivisions also contribute to the GABA source, although levels are much less than in the DNLL (Oliver, 2005).

GABAergic neurons in the IC are classified into various patterns, known as regular-sustained neurons and buildup neurons (Le Beau et al., 1996; Bal et al., 2002; Ono et al., 2005). This classification is based on neuronal responses after constant current injection (depolarization or hyperpolarization) (Peruzzi et al., 2000; Sivaramakrishnan and Oliver, 2001; Tan et al., 2007; Sun and Wu, 2008a). Electrophysiological studies have primarily focused on the distinction of ion channels in the various patterns, although the physiological role in acoustic information processing remains unknown.

In the present study, whole-cell patch clamp was used to classify IC neurons. The stimulation responses in the DNLL were recorded in neurons with various firing patterns, while the current was injected to the IC neuron to modify the discharge to a natural sound. The antagonist of the GABAergic receptor bicuculline was applied to validate the neurotransmitter responses. This study sought to find the relationship between electrophysiological patterns and acoustic processing.

Materials and Methods

Experimental animals

The animal studies were approved by the Committee for Institutional Animal Care and Use Committee of Institute of Beijing Chaoyang Hospital and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Precautions were taken to minimize suffering and the number of animals used in each experiment. Seven specific-pathogen-free Sprague-Dawley rats (13–17 days old, either sex) were provided by the Animal Service Center of Capital Medical University in Peking of China (license No. SCXK2011-0011).

Preparation of brain slices

Rat pups were decapitated following deep anesthesia with 4 mL ether. The brain was rapidly removed from the skull, transferred into ice-cooled artificial cerebrospinal fluid, and continuously saturated with 5% CO_2 and 95% O_2. The pH was adjusted to 7.3. The brain block was then cut into coronal slices of 300-μm thickness with a vibratome (VT1000S; Leica, Nussloch, Germany), which was filled with the cold artificial cerebrospinal fluid. The prepared slices, which contained the IC and DNLL, were incubated in oxygenated artificial cerebrospinal fluid at room temperature at least for 1 hour. One to two slices were prepared from each animal. After incubation, the individual slices were transferred to a recording chamber, and bathed with oxygenated artificial cerebrospinal fluid at 3–4 mL/min by gravity-fed perfusion at 31 ± 1°C. The slices were covered with a platinum-nylon net to prevent movement.

Artificial cerebrospinal fluid contained the following composition: 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM D-glucose, 2 mM $CaCl_2$, and 1.5 mM $MgCl_2$. NaH_2PO_4 was from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Solutions of 25 μM D-(-)-2-animo-5-phosphonopentanoic acid (D-AP5) and 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) were applied to normal artificial cerebrospinal fluid for the entire experiment. In some experiments, 10 μM bicuculline was used to verify $GABA_A$ receptor-medicated currents after recording action potential. All chemicals were supplied by Sigma (St. Louis, MO, USA).

Whole-cell patch clamp recording

Glass microelectrodes were used to record responses of injected current and evoked postsynaptic currents. The microelectrodes were prepared from thin-walled glass pipettes (GB 150F-8P, Sutter instrument, Novato, CA, USA) with a two-stage vertical puller (PC-10; Narishige Scientific Instrument Lab, Tokyo, Japan). The electrode resistance amounted to 4–6 MΩ after filling with intracellular solution. The internal solution contained the following composition: 140 mM K-gluconate, 2 mM $MgCl_2$, 10 mM HEPES, 8 mM KCl, 2 mM Na_2ATP, and 0.2 mM MgGTP. The pH was adjusted to 7.3 with KOH. All chemicals were provided by Sigma.

A light microscope (BX51WI; Olympus, Tokyo, Japan) and a 40× long-working distance objective (NA 0.8) were used to view the slices and to locate DNLL neurons and IC neurons. Whole-cell recording was performed with an EPC-10 double amplifier (HEKA Instruments, Lambrecht, Germany) on IC neurons. Fast capacitance, slow capacitance, and series resistance were compensated until the baseline remained straight at a membrane potential of –60

mV. All records were digitized using HEKA Patchmaster software (HEKA Elektronik, Lambrecht, Germany). The signals were filtered at 3 kHz.

Action potential recording of GABAergic neurons in IC
Whole-cell configuration was made in the current clamp mode and the holding current was set to 0 mA. The recording took place for at least 5 minutes after whole-cell configuration. In each recording, 100 pA current was injected into the neurons.

Action potentials of IC neurons after evoking ipsilateral DNLL neurons
A concentric stimulating electrode was placed on the DNLL. The synaptic response was evoked by square wave pulses of 100 μs duration and variable intensity. A certain threshold of stimulus intensity, below which no response was detected, was defined to record the evoked inhibitory postsynaptic currents. The recording stimulus intensity was maintained at 1.5 fold of this threshold. A stimulus series containing three single stimuli at a 500-μs interval was applied before 100-pA current inspiring action potentials. The duration of the series of pulses was 1,300 μs. The postsynaptic currents in IC neurons were subsequently recorded to ensure that the recorded inhibitory postsynaptic currents were mediated by the GABAergic receptor. The inhibitory postsynaptic currents mediated by the GABAergic receptor were diminished following application of bicuculline.

Action potential responses of IC neurons after extracellular application of bicuculline
The perfusion of bicuculline (10 μM; Sigma) in artificial cerebrospinal fluid allowed for observation of action potential changes in IC neurons under stimulation of DNLL neurons.

Data acquisition and analysis
All recordings were performed after applying whole-cell clamp patch and allowing the IC neuron membrane to reach a stable resting potential. Spikes were elicited by current injection to IC neurons. Changes in amplitude and firing frequency were measured after simulating in DNLL and application of the GABAA receptor antagonist bicuculline. Postsynaptic currents were measured after paired-stimulation to DNLL. Data were expressed as the mean ± SEM and were recorded and analyzed using the PatchMaster software.

Results

Seven neurons were analyzed. These neurons were located in the IC, according to the Paxinos and Watson atlas (Paxinos and Watson, 2005). All neurons were recorded using the whole-cell patch clamp method and values remained as stable membrane potentials (**Table 1**).

Identification of neuronal types according to discharge patterns
All tested IC neurons exhibited inhibitory discharges (*n* =

7). Classification was based on neuronal responses to continuous injections of depolarizing currents. All neurons exhibited sustained discharge to suprathreshold currents, and continuously fired over the entire period of injected current. The inter-spike intervals were measured, and the ratio of the last interval to the first interval was defined as the adaptation ratio (Sun and Wu, 2008b).

The tested neurons were classified into two types according to adaptation ratio. Four out of seven neurons exhibited continuous pulses with relatively constant inter-spike intervals (sustained-regular firing pattern; **Figure 1A**). The remaining neurons responded to continuous discharge as well, although the inter-spike intervals were prolonged, suggesting that the later interval was longer than the previous one (sustained-adapting neurons; **Figure 2A**). The average adaptation ratio for adapting neurons was 1.34 (ranging from 1.09–1.60), while the average adaptation ratio for regular neurons was 1.19 (ranging from 0.98–1.57; **Table 1**).

Pattern responses of IC neurons to stimulation in the ipsilateral DNLL
The stimulating electrode was placed in the area ventral lateral to the IC, where fibers from the DNLL projected to the IC. Continuous current was injected to imitate the response of IC neurons to acoustic stimuli (Peruzzi et al., 2000; Pecka et al., 2007). A short train of three pulses was then stimulated in the area.

One neuron with sustained-regular firing pattern showed suppressed responses to the stimulation, with a reduced frequency of discharge (**Figure 1B**). The responses of the remaining three neurons in this pattern were facilitated or slightly suppressed by the stimulation. In the sustained-adapting firing pattern, the responses of one neuron were obviously suppressed (**Figure 2B**), while the remaining neurons exhibited relatively constant responses.

The response width was presented by the action potential duration at 50%. The average action potential duration from 50% of neurons with sustained-regular pattern was greater than those with the sustained-adapting firing pattern, although both patterns exhibited an increased value after stimulation (**Table 2**).

Response pharmacology of two patterns in IC neurons
As mentioned above, two IC neurons with distinct firing patterns showed suppressed responses to the stimulation. Because the DNLL is the main origin of inhibitory projections to the IC and the majority of the fibers were GABAergic (Oliver, 2005), the GABAergic receptor antagonist bicuculline was applied. The amplitude of the sustained-adapting neuron increased to normal levels after bicuculline application, while amplitude of the sustained-regular neuron continued to decrease.

The responses to bicuculline in the remaining neurons were also recorded. The sustained-regular neurons exhibited increased responses, and responses of the sustained-adapting neurons became normal (**Figure 1C, 2C,**

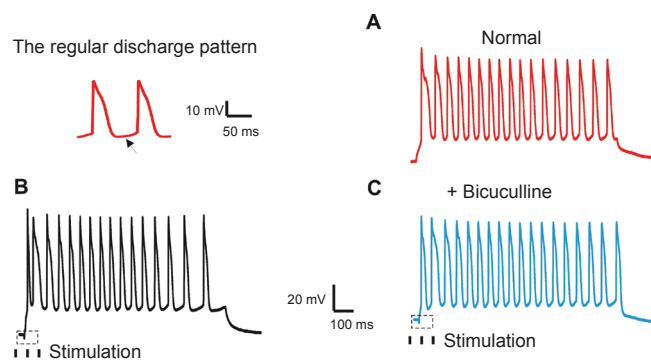**Table 1 Membrane properties of regular and adapting neurons**

Firing pattern	Amplitude (mV)	Threshold (mV)	Adaptation ratio
Regular	59.83±4.59	−43.29±0.83	1.19±0.13
Adapting	60.23±6.69	−40.53±0.36	1.34±0.15

Data are expressed as the mean ± SEM (*n* = 7 neurons).

Table 3 Adaptation ratio of neurons with two firing patterns

Firing pattern	Normal	Stimulation	Bicuculline
Regular	1.19±0.13	1.28±0.05	1.17±0.38
Adapting	1.34±0.15	1.46±0.18	1.99±0.65

Data are expressed as the mean ± SEM (*n* = 7 neurons).

Table 2 Action potential properties of neurons in the IC with two patterns after stimulation and application of bicuculline

Pattern	Amplitude (mV)	Action potential duration at 50% (ms)	Frequency (Hz)
Regular			
Normal	59.83±4.59	2.23±0.98	19.75±2.66
Stimulation	60.82±5.44	2.71±1.28	20.50±3.66
Bicuculline	59.91±4.66	4.49±2.24	22.00±5.28
Adapting			
Normal	60.23±6.69	1.47±0.62	16.67±4.06
Stimulation	57.33±7.77	1.54±0.67	15.67±3.18
Bicuculline	59.55±7.04	2.40±0.74	11.67±3.67

Data are expressed as the mean ± SEM (*n* = 7 neurons).

Figure 1 Responses of IC neurons with regular firing pattern.
(A) Normal responses: IC neurons exhibit sustained discharge after continuous injection of depolarizing current. The intervals are relatively constant (adaptation ratio = 1.19 ± 0.13). (B) Responses after stimulation: Amplitude of responses are suppressed, and discharge frequency is decreased. (C) Responses after application of bicuculline. Amplitude returns to a normal level, while frequency decreases. IC: Inferior colliculus.

Figure 2 Responses of IC neurons with adapting firing pattern.
(A) Normal responses: IC neurons exhibit sustained discharge after continuous injection of depolarizing current. The intervals are relatively increased (adaptation ratio = 1.34 ± 0.15). (B) Responses after stimulation: Amplitude of responses is facilitated, and the discharge frequency increases. (C) Responses after application of bicuculline: amplitude returns to a normal level, while frequency increases. The dashed frame represents stimulation on the dorsal nucleus of the lateral lemniscus. IC: Inferior colliculus.

Table 2).

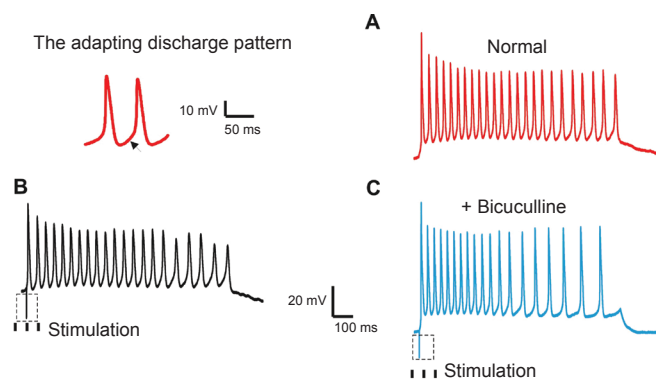**Adaptation ratio of IC neurons with two firing patterns**
The standards to identify the regular and adapting firing patterns were various in the previous studies. The first standard was the adaptation ratio. The second standard was the ratio of each following interval to the first interval, and if the following interval was as twice as the first interval, the neuron was identified as the adapting firing pattern. (Peruzzi et al., 2000; Sivaramakrishnan and Oliver, 2001; Bal et al., 2002; Ono et al., 2005; Tan et al., 2007; Sun and Wu, 2008a).

The present study chose the adaptation ratio to quantify the two firing patterns. The adaptation ratio in the sustained-regular firing pattern was relatively constant and was not affected by stimulation or bicuculline (**Table 3**). The adaptation ratio in the sustained-adapting firing pattern, however, exhibited an obvious increase following stimulation and bicuculline (**Table 3**), and the maximal value was twice the normal value.

Discussion
Classification of IC neurons
IC neurons with sustained-regular or sustained-adapting firing patterns were identified in the present study, which was consistent with previous studies (Oliver et al., 1994; Peruzzi et al., 2000; Sivaramakrishnan and Oliver, 2001). IC neurons are classified into flat or less-flat categories according to morphology of the shape and orientation of the dendritic tree (Oliver et al., 1994; Malmierca et al., 1995; Peruzzi et al., 2000; Winter and Schreiner, 2005). However, some physiological studies have suggested that identification of IC neurons by the firing pattern is possibly associated with their functions. Peruzzi et al. (2000) identified at least three physiological types of IC neurons. Sivaramakrishnan and Oliver (2001) extended the firing patterns to six types, including sustained-regular, sustained-adapting, onset, pause-buildup, rebound, and rebound regular, and these types were present not only in the central nucleus, but also in the cortex of the IC (Peruzzi et al., 2000; Sun and Wu, 2008a, b). Tan et al. (2007) performed similar experiments using *in vivo* patch-

clamp in the IC of rats and mice; results showed five patterns without the onset-firing pattern that were previously reported in *in vitro* studies. Tan et al. (2007) argued that this was due to the absence of recording the Kv1.1 potassium channel, which is thought to elicit the onset pattern.

A similar result was observed in a previous study of firing patterns of GABAergic neurons in the IC. Ono et al. (2005) labeled IC neurons with green fluorescent protein fluorescence to discriminate GABAergic from non-GABAergic neurons; the GABAergic neurons were classified into two distinct types according to responses to depolarized currents injection. The first type was a tonic-type neuron that exhibited sustained and regular firing (with slight adapting) to a long period of depolarized current injection, which was consistent with our results. The second type was a transient-type neuron with discharge in the beginning of current injection, which was not observed in our study.

Sivaramakrishnan and Oliver (2001) suggested that the various firing patterns in IC neurons was due to the distinct potassium ion channel on the neuronal membrane. Delayed-rectifying 4-AP-sensitive potassium currents result in sustained discharge in the sustained-regular firing pattern during stimulation, indicating that neurons provide information about input duration. The sustained-adapting firing pattern is determined by apamin and charybdotoxin-sensitive calcium-activated potassium currents, which possibly underlie the sensitivity of neurons to interaural phase modulation.

Although electrophysiological classification of IC neurons is associated with physiological function, the exact role of each pattern is not clear. The rebound-firing pattern is observed by preceding hyperpolarization before depolarized current injection. Some studies have shown that membrane excitability becomes modified after pre-hyperpolarization. Because IC neurons converge projections from lower or higher auditory nuclei, this modification was proposed as a possible mechanism for integrating excitatory and inhibitory projections (Kuwada and Batra, 1999).

GABA effects on responses of IC neurons

Although the average response of sustained-regular neurons was facilitated by stimulation in the DNLL, we noticed that one neuron of this type exhibited an evident reduction in amplitude (from 60.91 mV to 54.14 mV). This suppression, however, did not recover to a normal level after bicuculline application, indicating that GABAA was not acting as the transmitter.

The IC contains two types of inhibitory neurotransmitters – glycine and GABA (Nakamoto et al., 2014; Ono and Oliver, 2014). Glycine is produced mainly by the ipsilateral lateral superior olivary nuclei, and accounts for 26% of synaptic endings in the IC (Oliver, 2005). Because the lateral superior olivary nucleus is separated from the DNLL and there is no possibility to confuse these two nuclei, it meant that glycine cannot be the transmitter in our study. GABA, a vital inhibitory neurotransmitter in the IC, is mainly produced by the bilateral DNLL and intrinsic inputs from subdivisions in the

IC (Shneiderman et al., 1993; González-Hernández et al., 1996; Winter and Schreiner, 2005; Pollak et al., 2011; Orton and Rees, 2014; Sturm et al., 2014). Three types of GABA receptors have been described: GABA$_A$, GABA$_B$, and GABAc (Caspary et al., 2008). GABA$_A$ elicits hyperpolarization of membrane potential by opening the chloride channel to inhibit evoking of the action potential (Sun and Wu, 2008a). The GABA$_B$ receptor can act as presynaptic or postsynaptic receptor. The postsynaptic GABA$_B$ receptor elicits postsynaptic inhibitory potential and modulates membrane excitability, which is similar to GABA$_A$ functions. The presynaptic GABA$_B$ receptor modulates synaptic response mediated by GABA$_A$, suggesting that it modulates synaptic transmission (Ma et al., 2002; Sun and Wu, 2009; Jamal et al., 2012). Therefore, GABA$_B$ remains the most likely neurotransmitter, although we did not administer GABA$_B$ receptor agonist to verify this hypothesis.

In this study, a neuron with a sustained-adapting firing pattern exhibited obvious suppression to stimulation in the DNLL, with the amplitude ranging from 56.95 mV to 47.30 mV. However, the amplitude recovered to a normal level (54.32 mV) after application of bicuculline. These results were inconsistent with a previous study by Ono et al. (2005). The neuron in the present study exhibited a remarkable ability to adapt to the firing pattern (adaptation ratio = 1.608), which suggested that GABAergic neurons also exhibit sustained-adapting firing.

The majority of neurons in this study exhibited relatively constant discharge to stimulation and application of bicuculline, which was consistent with a previous study (Ono et al., 2005). The sustained property acts as a distinct function in input modulation to the IC. Tan et al. (2007) investigated the phase-locked responses of IC neurons with three different firing patterns to the signals; their results indicated that the sustained pattern exhibits a phase-locked discharge to the signal, and the membrane properties modulated by the delayed-rectifier potassium current dominate the phase-locked discharge, which is potentially the intrinsic mechanism.

Auditory function of GABAergic neurons in the IC

Previous studies have shown that GABA contributes to sound localization in the reverberant environment, especially in the precedence effect, which refers to the phenomenon of the auditory system to improve localization of initial sound by suppressing directional information of reflected sound (Wallach et al., 1949; Yang and Grantham, 1997; Litovsky et al., 1999; Li and Yue, 2002; Tollin et al., 2004; Pecka et al., 2007; Tolnai et al., 2014; Brown et al., 2015b). Some physiological studies in animals (*e.g.*, cats, rats, and rabbits) showed that most IC neurons exhibit suppression to lag responses in a short interstimulus delay, which is similar to human behavioral studies, and the suppression remains obvious even if the lead does not elicit responses (Yin, 1994; Fitzpatrick et al., 1995, 1999; Litovsky et al., 1997; Litovsky and Yin, 1998a, b; Litovsky and Delgutte, 2002; Dent et al., 2009; Song et al., 2011; Wang et al., 2014). The suppression takes place as a

long-lasting inhibition (as long as 30 ms), which exceeds the refractory period of IC (Yin, 1994; Fitzpatrick et al., 1995; Litovsky and Yin, 1998b; Bauer et al., 2000). These results indicate that inhibition, rather than the refractory period, causes suppression to lag in the IC (Litovsky and Yin, 1998b; Litovsky et al., 1999). Several *in vivo* studies have shown that phenobarbital and GABA prolong the half maximal interstimulus delay to paired-stimuli and reduce the peak of responses to lag in the rat IC, indicating that suppression to lag is enhanced by GABA (Covey et al., 1996; Kidd and Kelly, 1996; Tollin et al., 2004; Song et al., 2011; Wang et al., 2014; Brown et al., 2015a).

The long-lasting inhibition refers to a phenomenon where, IC neurons show an inhibitory effect that persists for milliseconds beyond the end of the leading stimuli when a paired lead-lag sound is performed. This has been observed in some *in vivo* physiological studies that recorded discharge of a single unit to a paired-sound separated by various interaural time differences and interaural intensity differences in different mammals (bat, rabbit, and cat). Results showed that IC neurons exhibit long-lasting inhibition that exceeds the period of the leading sound, and this suppression is affected by the duration and relative intensity of paired stimulus (Li and Kelly, 1992; Yin, 1994; Fitzpatrick et al., 1995; Covey et al., 1996; Li et al., 1998; Litovsky and Yin, 1998b; Wang et al., 2014). Similar findings have been observed in whole-cell patch clamp in conscious bats, where inhibition beyond the end of the sound stimuli lasted for ~27 ms (Covey et al., 1996).

Although the origin of long-lasting inhibition is not well understood, the possibilities are discussed below. Similar to previous studies, the inhibition observed in the present study was eliminated by bicuculline, a blocker of GABAergic receptor, indicating that GABA is at least partially responsible for the generation of long-lasting inhibition. In accordance with the multiple sources of GABAergic projections to the IC, we will discuss three possibilities for the generation of long-lasting inhibition.

1) The IC contains three subdivisions that include the central nucleus of the IC, the dorsal cortex of the IC, and the external cortex of the IC; the bilateral IC connects each of these with the commissure of the IC (Covey et al., 1996; Li et al., 1998; Pollak et al., 2011). The inhibitory projections are GABAergic, although the functions of these intrinsic connections remain poorly understood. In the present study, we blocked the GABA receptors in a whole brain slice. Therefore, it cannot be ruled out that intrinsic GABAergic projections are involved in the generation of long-lasting inhibition.

2) The bilateral DNLL is connected by the commissure of Probst. Physiological studies have recorded persistent inhibition from the DNLL that lasts for tens of milliseconds and originates in the contralateral DNLL. Pecka et al. (2007) suggested that persistent inhibition contributes to the mechanisms underlying the precedence effect. This persistent inhibition lasts for milliseconds, which is similar to the duration of long-lasting inhibition observed in the previous studies, indicating that long-lasting inhibition likely comes from persistent inhibition in the DNLL.

3) Kidd and Kelly (1996) investigated the contribution of the DNLL to *in vivo* binaural responses in the rat IC. By testing different neural responses in the IC with various interaural time differences, they found that long-lasting inhibition was elicited by ipsilateral sound stimulation, which lasted for 10–20 ms in different neurons and was possibly affected by sound intensity. Kynurenic acid was then injected in the contralateral DNLL to block the N-methyl-D-aspartic acid (NMDA) receptor, resulting in reduced long-lasting inhibition. However, this suppression was not observed after injection into the ipsilateral DNLL, indicating that NMDA contributes to long-lasting inhibition only in the IC. They then compared changes before and after administration of excitatory amino acid receptor antagonists, resulting in similar results to a previous study (Chen et al., 1999). Chen et al. suggested that NMDA contributes to extended GABA release in the DNLL, which subsequently increases inhibition to the contralateral IC. Together, these studies suggest that GABAergic projections from DNLL to the ipsilateral IC mediate long-lasting inhibition, and GABA release is modulated by NMDAergic projections from the contralateral DNLL. Further studies are needed to support this hypothesis.

Although the responses of two IC neurons were suppressed by stimulation in the ipsilateral DNLL, an obvious inhibition that lasted for milliseconds was not recorded. There are three possible reasons for this. First, the simple volume was small in this study, and we recorded responses of IC neurons with only two firing patterns. It is possible that the other types of neurons accounted for the cellular basis of long-lasting inhibition. For example, the rebound-firing pattern exhibits discharge after depolarization, which is similar to a neuron that receives inhibitory inputs preceding excitatory inputs. The rebound neuron is critical for controlling spiking patterns, and possibly underlies the encoding of acoustic temporal information (Malmierca et al., 1995). Second, we chose the incorrect site for stimulation, and this was subsequently excluded. Prior to recording, we stimulated the site and recorded the inhibitory currents. These currents were diminished by bicuculline. Third, although the IC receives projections from the bilateral DNLL, some physiological studies suggest that the contralateral DNLL is the major origin of GABAergic input fibers of the IC. We stimulated the contralateral DNLL, but did not record the IC responses. It is possible that the commissure connecting the bilateral DNLL was not included in the brain slice or was damaged during preparation.

In conclusion, two types of IC neurons were observed, including the sustained-regular and sustained-adapting firing pattern. One neuron from each type exhibited a suppressed response to stimulation in the ipsilateral DNLL, and responses of the neuron with the adapting firing pattern recovered to a normal level, suggesting that GABAergic neurons exhibit sustained-adapting firing. Further pre-hyperpolarization studies are needed to observe neurons with other firing patterns. Responses of neurons with different firing

patterns may indicate a physiological relation to acoustic stimuli, especially directional information.

Author contributions: *XTL carried out the experiment, analyzed the data, graphed the figures, and wrote the paper. NYW served as a principle investigator, designed the study and obtained the funding. YJW participated in data acquisition and analysis. ZQX and YFB were responsible for the design and performance of electrophysiological experiment. JFL, JSD and JYZ participated in data collection and analysis. All authors approved the final version of the paper.*

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Plagiarism check: *This paper was screened twice using Cross-Check to verify originality before publication.*

Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

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