Published in final edited form as:

Hear Res. 2020 January ; 385: 107844. doi:10.1016/j.heares.2019.107844.

Neuronal Sensitivity to the Interaural Time Difference of the Sound Envelope in the Mouse Inferior Colliculus.

Munenori Ono^{1,2}, Deborah C. Bishop¹, Douglas L. Oliver¹

¹Department of Neuroscience, University of Connecticut Health Center, Farmington, CT, USA
06030-3401

²Department of Physiology, Kanazawa Medical University, Ishikawa 920-0293, Japan

Abstract

We examined the sensitivity of the neurons in the mouse inferior colliculus (IC) to the interaural time differences (ITD) conveyed in the sound envelope. Utilizing optogenetic methods, we compared the responses to the ITD in the envelope of identified glutamatergic and GABAergic neurons. More than half of both cell types were sensitive to the envelope ITD, and the ITD curves were aligned at their troughs. Within the physiological ITD range of mice ($\pm 50 \mu\text{s}$), the ITD curves of both cell types had a higher firing rate when the contralateral envelope preceded the ipsilateral envelope. These results show that the circuitry to process ITD persists in the mouse despite its lack of low-frequency hearing. The sensitivity of IC neurons to ITD is most likely to be shaped by the binaural interaction of excitation and inhibition in the lateral superior olive.

Keywords

Interaural time difference; envelope; inferior colliculus; mice; glutamatergic and GABAergic neurons.

1. Introduction

Interaural time difference (ITD) is a critical binaural cue for sound localization for animals with a large head. ITD systematically varies dependent on the sound azimuth, and gives information about sound location to a listener. To utilize ITD for sound localization, the medial superior olivary nucleus (MSO) must code the phase difference between the sounds in two ears. However, phase-locking in the auditory nerves is limited to $<4 \text{ kHz}$ (Rose et al., 1967). Thus, the ITD in the ongoing fine structure of sound is only available for localization

Corresponding author: Dr. Munenori Ono, Department of Physiology, Kanazawa Medical University, Uchinada, Kahoku, Ishikawa, 920-0293 Japan. onomn@kanazawa-med.ac.jp.

AUTHOR CONTRIBUTIONS: MO and DLO designed the study; MO collected and analyzed the physiological data; DCB performed the histology, collected, and analyzed the anatomical data; MO and DLO wrote the paper.

The authors declare no competing financial interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

at frequencies where phase locking is present. Generally, the animals with a small head have poor sensitivity to low frequency sounds (< 1kHz).

Small animals, including mice, have a small head size and are thought to have only a small physiological range of ITD (e.g. $\pm 50 \mu\text{s}$ in mice; Allen et al., 2010). Instead, small animals use interaural level difference (ILD) as the predominant cue for sound localization. This is primarily coded in the lateral superior olivary nucleus (LSO). In line with this, small mammals such as mice and rats only hear high frequency sounds which generate larger ILDs than low frequency sounds (Grothe et al., 2014), and it has been reported that they could not utilize ITD in low-frequency sounds as a sound localization cue (Allen et al., 2010; Heffner, 2001).

ITD is not only conveyed in the fine structure of sound but also in the envelope of the sound. The envelope is the contour of the amplitude modulation (AM) of the sound, and the rate of modulation in natural sound can be low (e.g. from 2Hz to 300 Hz in human speech) (Dietz et al., 2013). Previous psychophysical studies have shown that human listeners use the ITD in the envelope as a sound localization cue as well as the ITD in the fine structure (Bernstein et al., 1994; Bernstein et al., 2002; Bernstein et al., 2008; Bernstein et al., 2012; Klein-Hennig et al., 2011; McFadden et al., 1976; Nuetzel et al., 1976). Furthermore, behavioral studies suggested that some animals also localize the sound using the ITD in the envelope (Heffner et al., 2001; Heffner et al., 2015; Keating et al., 2013; Li et al., 2019). The neural basis of envelope ITD coding begins with the auditory nerve that is well-synchronized to AM sound in this range of modulation (Joris et al., 2004). This synchronization is preserved in the auditory nuclei in the brainstem where the MSO and LSO can detect the ITD in the envelope and send information about the envelope to the inferior colliculus (IC). Since small mammals have a well-developed LSO, envelope ITD processing utilized for sound localization also might occur in their auditory pathways. Indeed, previous studies reported that the ITD in the envelope were detected in the LSO of rabbit (Batra et al., 1997c) and cat (Joris et al., 1995). Furthermore, previous studies on the LSO of gerbil (Beiderbeck et al., 2018; Franken et al., 2016) and bat (Park et al., 1996) have shown that LSO neurons were quite sensitive to ITDs. Although the ITD sensitivity of LSO neurons in mice has not been examined, an *in vitro* study showed that the activity of the LSO neurons in the slice preparations of mice was sensitive to small differences in bilateral timing of synaptic inputs (Wu et al., 1992a). These evidences suggest that the envelope ITD is likely to be processed in the auditory pathways of small mammals. However, the neuronal response to the ITD in the envelope of AM sounds more typical of natural sounds or communication sounds remains to be studied in small mammals.

Here, we examined the neuronal sensitivity to envelope ITD in mice, the most useful laboratory animal for genetic engineering. Responses evoked by binaural AM sounds were recorded from the IC, an auditory center in the midbrain that receives and integrates the afferent inputs from virtually all nuclei in the auditory brainstem. The IC consists of glutamatergic (75 %) and GABAergic neurons (25%). In the central sensory pathways, the interaction of excitation and inhibition is known to be critical in shaping the neural responses; however, it is not known to what extent these neuron types process ITD. To distinguish the glutamatergic and GABAergic neurons in *in vivo* recordings, we used

transgenic mice (VGAT-ChR2 mice) in which the inhibitory neurons specifically express channelrhodopsin2 (Zhao et al., 2011). As shown in our previous studies, the laser-light stimulation of the IC evoked firing in GABAergic and inhibited firing in glutamatergic neurons (Ono et al., 2016; Ono et al., 2017). Using this method, we compared the sensitivity of glutamatergic and GABAergic neurons in the IC to the ITD in the envelope. Our results reveal envelope ITD sensitivity in the mouse in both cell types. In most neurons, the ITD curves were aligned at their troughs, as was seen in the LSO neurons. Moreover, within the physiological ITD range of mice ($\pm 50 \mu\text{s}$), the ITD curves of both cell types had higher firing rates when the contralateral envelope preceded the ipsilateral envelope. These results suggested that envelope ITD sensitivity in the mouse IC might be shaped by the binaural interaction of the excitation and inhibition or inherited from the LSO neurons, and it might enhance the responses in IC induced by ILD in both glutamatergic and GABAergic neurons.

2. Materials and Methods

2.1 Animals.

We used 30 transgenic mice (VGAT-mhChR2-YFP, Tg(Slc32a1-COP4*H134R/EYFP)8Gfng/J; #14548, Jackson Labs; here referred to as VGAT-ChR2 mice) of either sex (Postnatal day 1.5 – 4 months). All experiments were approved by the Animal Care and Use Committee at the University of Connecticut Health Center and done in accordance with institutional guidelines and with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

2.2 Surgery

Before the experiment, VGAT-ChR2 mice were anesthetized with a mixture of ketamine (100 mg/kg), xylazine (20 mg/kg) and acepromazine (10 mg/kg), and maintained in an areflexive state with isoflurane (0.5 - 1 %) mixed with oxygen during the surgery and recording. Body temperature was monitored and maintained at $> 35^\circ\text{C}$ by a DC temperature controller (FHC, Bowdoin, ME). Vital signs also were monitored (MouseOx Plus, Starr Life Science Corp, PA), and the surgery and recordings were done in a double-walled sound attenuating chamber (IAC, Bronx, NY). The surgical procedure was described previously (Ono et al., 2014). After the craniotomy, the auditory brainstem response (ABR) to a click (0.5 ms) was measured to verify normal hearing. The threshold of the ABR was (left, 30.7 ± 1.1 dB, right, 30.3 ± 1.0 dB, $n = 30$). Mice were used for experiments only when the ABR threshold was less than 40 dB.

2.3 Sound system.

Acoustic stimuli were generated by a TDT System 3 (TDT, Tucker Davis Technologies, Alachua, FL) under the control of custom software (Brian Bishop, UCHC) written in MATLAB (Mathworks, Portola Valley, CA). All sounds were delivered by a closed system that included electrostatic speakers (TDT EC1) coupled to small metal tubes inserted into the external auditory meatus to minimize the acoustic crosstalk (Ono et al., 2014). The sound system was calibrated from 100 – 100000 Hz by using a 1/4" microphone (Type 4135, Brüel & Kjaer, Naerum, Denmark) at the end of the ear tubes.

2.4 Single unit recordings with optogenetic identification of cell types.

The procedure for the single unit recordings with optogenetic cell type identification was described previously (Ono et al., 2016). Briefly, single cell extracellular recordings were obtained using borosilicate glass pipettes filled with 0.01M phosphate buffered saline (pH 7.4) with 2 % Neurobiotin (4 – 7 M Ω). After the single unit was isolated, we identified the cell type optogenetically. Light was generated by a blue laser (MBL-III-473nm - 200 mW, CNI, China) and delivered through a 400 μ m-diameter optical fiber whose tip was placed several millimeters above the brain surface. The light stimulus was a 10 - 50 mW/mm² light pulse of 30 ms at the fiber tip. Light pulses were given every four seconds. Light evoked firing in GABAergic neurons but suppressed spikes in other neurons. We judged that the neuron was suppressed when the light reduced sound evoked spikes by more than 50%. We verified the validity of the optogenetic identification by immunohistochemical identification of GAD67 (Ono et al., 2016).

The signals were amplified, bandpass filtered from 300 to 4000 Hz, and sampled at 10 kHz with a Multiclamp 700B Amplifier, Digidata 1440A digitizer and Clampex 10.2 system (Molecular Devices). The voltage signals were recorded in current clamp mode. In parallel with recording the signals, the spike times were extracted using a window discriminator and recorded with the TDT System 3 and MATLAB software.

2.5 Acoustic stimuli.

After the cell type identification, the neuron's best frequency (BF) was determined by 100 ms contralateral tone bursts at 70 - 80 dB (5 ms rise/fall). First, we presented the pure tones whose frequency ranged from 2 to 76.1 kHz (0.25 octave step, 3 times), and then presented tones with small frequency steps within a narrower frequency range. We used 500 Hz or 1 kHz steps when the BF was below or above 20 kHz, respectively. The characteristic frequency (CF) and threshold were also determined by repeating the process as lower sound intensities (5 dB step). BF was defined as the frequency where the neuron showed the strongest response. CF was defined as the frequency where the lowest sound level could evoke the response. In two glutamatergic neurons, we could not identify either BF or CF because their responsiveness to tones was too low.

To measure the neuronal sensitivity to the ITD in the envelope, we used a binaural 'beat' AM sound in which a 60 - 70 dB AM one-octave noise (5.1 s every 10s, presented five times) was modulated at different rates but at the same SPL level in the two ears. In the stimulus, the modulation frequency of the ipsilateral side was set higher than that of contralateral side by 1 Hz. The center frequency of the one-octave noise was set at CF (GABAergic neurons, 20.7 ± 1.2 Hz, $n = 18$, glutamatergic neurons, 22.9 ± 1.2 , $n = 27$) in both ears. In two glutamatergic neurons, broad-band noise (4 - 40 kHz) was used instead of the one-octave noise because CF could not be identified in these cells.

The noise had the same waveform with the same phase in both ears. Thus, there was no phase difference in the noise delivered to both ears. Consequently, in the binaural beat AM sound that we used, ITD was only generated in the envelope, and not in the fine sound

structure. The ITD sensitivity in envelope was assessed by varying the modulation frequency from 32 to 512 Hz (the values of the contralateral side) by one octave step.

As a modulation envelope, we used either a sinusoid or a raised sine envelope (Bernstein et al., 2009). The raised sine stimulus is represented by the formula as follows:

$$Y(t) = N(t)(2((1 + \sin(2\pi f_m t))/2)^n),$$

where $N(t)$ is the one-octave noise, f_m is the modulation frequency and n is the roll-off factor. As n is larger, the envelope becomes steeper. We used the raised sine stimuli with a roll-off factor of 16. The raised sine AM sound contains more spectral sidebands than SAM (Bernstein et al., 2009) as does any sound with a rapid rise time. Since the spectrum width of the carrier noise was shown to affect the sensitivity of the envelope ITD in LSO neurons, the broadening of the spectrum in the raised sine stimuli might affect the ITD sensitivity in the IC neurons. To validate the effect, we measured the spectrum of modeled waveforms and found that the raised sine stimuli had a broader spectrum band than one-octave noise as the modulation frequency increased. However, the broadening of the spectrum band was less than 10% even at a modulation frequency of 512 Hz when the roll-off factor was 16 (data not shown). So, the effect of the spectrum change is likely to be limited.

In addition to the binaural stimuli, we tested the responses to the monaural AM sound in contralateral ear. The monaural AM sound was 60 - 70 dB, AM one-octave noise with the modulation envelopes of a sinusoid or a raised sine.

2.6 ITD sensitivity analysis.

ITD sensitivity of the recorded neuron was assessed by testing whether the spike responses to binaural beat AM sound was significantly synchronized to beat frequency (1 Hz). Among the responses whose firing rate was higher than 1 Hz, the synchronization was evaluated by the Rayleigh test ($p < 0.001$) (Yin et al., 1983a). The first 100 ms of the response was excluded from all the analysis of AM sound to avoid the effects of transient onset responses. To calculate the percentage of the neurons synchronized to beat frequency, we counted the number of neurons that showed significant synchronization to at least one modulation frequency in all the test modulation frequencies (32, 64, 128, 256 and 512 Hz). We called these neurons 'beat-sensitive neurons'. When the percentage of the beat-sensitive neurons was measured, we excluded the data from the neurons whose responses were not examined to all the test modulation frequencies. In other analyses on beat sensitivity, we included the data from the neurons to which all the test modulation frequencies were not examined.

Using the significantly synchronized response (Fig. 1A), we constructed the ITD sensitivity curve (Batra et al., 1993). The spike responses to each cycle of the beat frequency were divided into 10 bins, and the responses to the same interaural phase difference (IPD) were averaged to make a histogram (Fig. 1B). The averaged responses were plotted against IPD on either side of zero. Then the IPD was converted to the equivalent ITD (Fig. 1C, D). We constructed ITD curves from the responses to different modulation frequencies. To evaluate the shape of the ITD curve, we measured the peak and trough times of each curve. The peak

and trough time were measured as the ITDs closest to zero, at which the firing rate was maximum and minimum, respectively. These parameters were measured between -5 ms and 5 ms of ITD.

For the neurons in which the responses to all the test modulation frequencies were recorded, we constructed rate modulation transfer function (rMTF). The shape of rMTFs was evaluated by the method described in our previous work (Ono et al., 2017). Briefly, we first normalized an rMTF by its maximum firing rate. Then, we set three boundaries, 0.25, 0.5, 0.75, and checked if the normalized firing rate crossed the boundaries when the modulation frequency increased by an octave step. By the pattern of the transitions, we classified rMTF into six categories: low pass, high pass, band reject, band pass, all pass, multipeak (Fig. 3) (Ono et al., 2017). We excluded the neurons with low maximum firing rate to AM sound (<5 Hz) from shape evaluation and classified them as low rate.

For the neuron in which more than three ITD curves were recorded, we measured its characteristic phase (CP) and characteristic delay (CD) (Fig. 6). To measure the CD and the CP, we plotted the mean interaural phase of the response against the modulation frequency, and fitted the phases with linear regression (Batra et al., 1989; Batra et al., 1993; Kuwada et al., 1987; Yin et al., 1983b). The mean interaural phase was calculated by averaging the phases of the all the spikes in the response to the beat AM sound with each modulation frequency. The CP and the CD were measured as the intercept of the line with the ordinate and the slope of the line, respectively. The Data were analyzed with Clampex 10.2, MATLAB and Origin 2015.

2.7 Histology.

The histological procedure was described previously. Briefly, after recording a single cell, the recording site was marked with Neurobiotin by current injection (200 nA, 50% duty cycle of 500 ms, 5 min). When the electrophysiological experiment was finished, animals were given additional anesthesia (ketamine/Xylazine/Acepromazine, 200mg/kg+40mg/kg +20 mg/kg) and were transcardially perfused with 0.01M phosphate buffered saline (pH 7.4) followed by 4% paraformaldehyde (PFA) in 0.1 M buffer, pH 7.4. After dissection, brains were postfixed in 4% PFA and then stored in 30% sucrose solution. To identify the recording location and subregions of the IC, brains were sectioned at $40\text{ }\mu\text{m}$ and stained immunohistochemically. We used primary antibodies for GAD67 (Mouse anti-GAD67, Millipore MAB5406, 1:3000) and GLYT2 (Guinea pig anti-GLYT2 Millipore AB1773, 1:10000). After washing, the sections were reacted with secondary antibodies and streptavidin (Invitrogen Streptavidin AF568, 1 mg/mL). Secondary antibodies were AF488 goat anti-mouse (1:200) and AF647 goat anti-guinea pig (1:200). After staining, the sections were mounted, cover slipped and imaged with a Zeiss Axiovert 200M microscope (Carl Zeiss). The recording locations were identified by the Streptavidin AF568 signals in the sections. When multiple neurons were recorded in an animal, the location of each neuron was based on the recording depth and the mediolateral and anteroposterior position of the neuron in the IC. We excluded the data when the correspondence between the recording depth and the labeled site was ambiguous.

3. Results

3.1 Over half of GABAergic and glutamatergic IC neurons showed ITD sensitivity

We recorded the responses to binaural beat AM sound from 47 neurons, of which 18 were GABAergic and 29 were glutamatergic. ITD-sensitive neurons were found in the central nucleus of IC (ICC) in three regions: glycine-rich Area 1, GABA-rich Area 2 and the border of Area 1 and 2 (Choy Buentello et al., 2015; Ono et al., 2016). In addition to ICC, some neurons were found in the lateral cortex and a region ventral to ICC (Ono et al., 2017) (Table 1).

More than half of both GABAergic and glutamatergic neurons showed synchronization to the binaural beat frequency in envelopes (Fig. 1, Fig. 2A). When a neuron was synchronized to the beat frequency in envelopes, its response fluctuated with the beat frequency (1 Hz, Fig. 1A). The synchronization of the responses was evaluated by the Rayleigh test, and the ITD curve (Fig. 1C) was constructed from the IPD histogram (Fig. 1B, see Material and Methods). As a modulation envelope, we used a raised sine (raised) as well as a conventional sinusoid (SAM, Materials and methods). When the modulation frequency of the SAM tone varied from 32 Hz to 512 Hz, about half of the neurons of both cell types were synchronized to the binaural beat when modulation frequency was 64 Hz (Fig. 2B, left panel). As the modulation frequency of the SAM increased, fewer neurons of both types were synchronized to the beat frequency (Fig. 2B). The synchronization change with the modulation frequency was significant in the glutamatergic neurons ($p = 0.00545$, Fisher's exact test), but not in the GABAergic neurons ($p = 0.1892$, Fisher's exact test). The percentages of the neurons synchronized to the beat frequency were not significantly different between glutamatergic and GABAergic neurons ($p = 0.0625$, paired Wilcoxon signed-rank test). The glutamatergic neurons showed similar patterns of synchronization to raised sine stimuli, although fewer neurons were synchronized overall (Fig. 2B, right panel). The change with the modulation frequency did not reach the significant level ($p = 0.4944$, Fisher's exact test). In contrast, GABAergic neurons did not appear to prefer a particular modulation frequency when raised sign was used (Fig. 2B, right panel, $p = 1.0$, Fisher's exact test). Fewer GABAergic neurons were synchronized than glutamatergic neurons but this was not a significance difference ($p = 0.125$, paired Wilcoxon signed-rank test).

The neuronal ITD sensitivity is obtained by the coincidence detection of the binaural neural inputs that are synchronized to the fluctuation of sound. In the superior olivary complex (SOC), the responses of the ITD-sensitive neurons were synchronized to both the monaural modulation frequencies and the beat frequency (Batra et al., 1997b; Yin et al., 1990). Furthermore, in these neurons, the synchronization to the monaural stimuli was stronger than the interaural synchronization because the summation of the binaural inputs broadened the phase distribution of the responses (Batra et al., 1997b). To examine how the ITD-sensitive neurons in the IC were synchronized to the beat and monaural modulation frequencies in envelope, we quantified the phase locking of spike responses to sound stimuli by measuring vector strength (VS). VS of spike responses was measured to both beat (VS_{beat}) and contralateral modulation frequencies (VS_{contra}) in the ITD-sensitive neurons. They are shown in Figures 2C (SAM) and 2D (raised sine). In both cell classes and envelope

shapes, the VS_{contra} decreased as the modulation frequency increased (Fig. 2C, D). In contrast, the changes in VS_{beat} were non-monotonic when the modulation frequency varied (Fig. 2C, D). The VS_{beat} and VS_{contra} were hardly or only weakly correlated (SAM-GABA, $R = 0.178$, $n = 25$, SAM-GLU, $R = 0.208$, $n = 45$, Raised-GABA, $R = -0.316$, $n = 11$, Raised-GLU, $R = 0.112$, $n = 33$). As was shown in the SOC, the VS_{contra} was larger than the VS_{beat} when the modulation frequency was lower than 128 Hz (Fig. 2C, D). However, in most responses, the VS_{contra} was smaller than the VS_{beat} when the modulation frequency was higher than 256 Hz. These results suggested that the ITD sensitivity to the binaural beat with high modulation frequency was not likely to be shaped by the coincidence detection in the IC neurons.

3.2 rMTF to binaural beat AM.

The firing rate of the ITD-sensitive IC neurons also varied depending on modulation frequency. We measured the rMTF to binaural beat AM sound to evaluate the change of firing rate relevant to modulation frequency (Fig. 3). We classified rMTF into seven categories: low pass, high pass, band reject, band pass, all pass, multipeak and low rate (Materials and methods). Most neurons were classified as low rate, low pass or band reject in the response to SAM, and this was true for both GABAergic and glutamatergic neurons (Fig. 3). In the responses to raised sine, fewer neurons were classified into band reject (Fig. 3C, H) and more glutamatergic than GABAergic neurons were classified as high pass or others (band pass, all pass and multipeak), but these differences were not significant (GABA vs GLU, $p = 0.1105$. SAM vs raised sine, $p = 0.2073$. two-way ANOVA).

To examine the difference of overall firing rates between the GABAergic and glutamatergic neurons, the firing rates of all the recorded neurons were plotted against modulation frequency (Fig. 4). The synchronized and unsynchronized responses were compared. Synchronization was evaluated by Rayleigh test ($P < 0.001$). In response to SAM tones (Fig. 4A), the firing rates of synchronized glutamatergic neurons were significantly higher than the unsynchronized responses of both GABAergic and glutamatergic neurons at the modulation frequencies of 64 ($P < 0.05$, Tukey HSD test) and 128 Hz ($P < 0.01$, Tukey HSD test, Fig. 4A). The firing rates of synchronized glutamatergic neurons seemed to extend to higher frequencies than the rates of synchronized GABAergic neurons ($P > 0.05$, Tukey HSD test). In the responses to raised sine, this trend was unclear (Fig. 4B). For 32, 64, and 128 Hz modulations, synchronized GABAergic neurons fired at the same or higher rate as synchronized glutamatergic neurons. At 128 Hz, the firing rate of the synchronized responses of both cell types were significantly higher than that of unsynchronized neurons of both cell types ($P < 0.01$ or $P < 0.01$, Tukey HSD test, Fig. 4B). These results suggested that some glutamatergic neurons in the IC may transmit ITD information with a high firing rate. Further, they showed that the GABAergic neurons in the IC can send either synchronized or unsynchronized inhibitory outputs to the postsynaptic neurons depending on the characteristics of the acoustic stimulus.

3.3 The envelope ITD curves of most IC neurons in the mouse were aligned at troughs.

Previous studies reported that two types of ITD sensitivity were observed in the IC of the rabbit: peak-type and trough-type (Batra et al., 1993). In the trough type neurons, the ITD

curves are aligned at troughs (Fig. 1C, D, 5A, B) to produce a composite (average ITD) curve with a distinct trough. The ITD curves in the peak-type neuron are aligned at peaks (Fig. 5D). Some composite curves have both a distinct trough and a distinct peak (Fig. 5C).

To examine the pattern of the ITD sensitivity in the mouse IC, we calculated the characteristic phase (CP) and characteristic delay (CD, Materials and methods, Fig. 5E-H). The CD and the CP were measured by fitting the relationship between the mean interaural phase of the response and the modulation frequency with a linear regression (Fig. 5E). CP is the index of the alignment: A CP of 0 indicates that the ITD curves are aligned near their peaks, while a CP of 0.5 indicates that the ITD curves are aligned near their troughs (Batra et al., 1993; Kuwada et al., 1987; Yin et al., 1983b). In the both GABAergic and glutamatergic neurons, CPs were close to 0.5 (Fig. 5E-G), both to SAM (GABA, 0.46 ± 0.02 , $n=5$, GLU, 0.50 ± 0.01 , $n=9$) and raised sine stimuli (GABA, 0.41 , $n=1$, GLU, 0.46 ± 0.03). This observation is consistent with the trough-type response of most neurons in the mouse IC. The other parameter, CD, gives the common ITD at which maximal excitation or suppression occurs in peak- or trough- type neuron, respectively. To examine if the CDs correspond to trough or peak, we plotted the CD against mean trough time or peak time (Fig. 5H). In most responses, the mean trough time was close to CD (Fig. 5H, left panel), while the mean peak time was not (Fig. 6D, right panel). To compare the proximity to CD, we calculated the squared deviation of each point from the unity line (Fig. 5H, dotted line) in the scattered plot of mean trough time and peak time. The squared deviation was significantly smaller in mean trough time than in mean peak time (trough, $0.87 \pm 0.38 \text{ ms}^2$, peak, $6.62 \pm 1.31 \text{ ms}^2$, $n=21$, $P=0.00061$, Wilcoxon signed rank test). These results showed that CD was close to trough in the most IC neurons. In addition, we analyzed the trough and peak of the individual ITD curves (Fig. S1), and this also showed that the most ITD curves were aligned at their troughs. It suggested that suppression might occur at a common ITD to shape the trough type responses (Grothe et al., 2014).

3.4 The ITD-evoked response was stronger when the contralateral sound preceded the ipsilateral sound.

The physiological ITD of mice is small ($\pm 50 \mu\text{s}$) because of their small head size ($\sim 16 \text{ mm}$, Allen et al., 2010). It has been proposed that the ITD information in small animals might be coded with the slope of the ITD curve within the range of the physiological ITD (Brand et al., 2002; McAlpine et al., 2001). In Figure 6A (SAM) and 6B (raised), the normalized ITD curves are presented in the range of -0.2 ms to 0.2 ms . In most ITD curves, the slope of the ITD curve within the physiological ITD range (gray filled square) was positive toward contralateral sides, regardless of the cell type and stimulation type (Fig. 6A, B). To evaluate the distribution of the slope quantitatively, we measured the slope of the ITD curve around zero ITD (Fig. 6C, D). The positive value of the slope means that the response was stronger when the contralateral sound preceded the ipsilateral sound. In both GABAergic and glutamatergic neurons, most neurons had positive slope values (Fig. 6C, D). The slope was slightly higher, but not significantly so, in the responses to the raised sine (GABA, $15.2 \pm 11.3 \text{ Hz/ms}$, $n=12$, GLU, $17.7 \pm 7.6 \text{ Hz/ms}$, $n=32$) as opposed to the SAM (GABA, $4.6 \pm 2.6 \text{ Hz/ms}$, $n=27$, $9.2 \pm 3.5 \text{ Hz/ms}$, $n=47$) ($P=0.117$, Two-way ANOVA test). The slope was not significantly different between the GABAergic and glutamatergic neurons, either (P

= 0.519, Two-way ANOVA test). Even when the slopes were normalized (GABA-SAM, 0.23 ± 0.07 /ms, n = 27, GABA-raised, 0.04 ± 0.20 /ms, n = 12, GLU-SAM, 0.11 ± 0.05 /ms, n = 47, GLU-raised, 0.22 ± 0.07 /ms, n = 32), they were not different between either the cell types (P = 0.840, Two-way ANOVA test) or the envelope shapes (P = 0.878, Two-way ANOVA test). Interestingly, this firing pattern shaped by envelope ITD matches that of the ILD, because in the IC, the ILD also evokes a higher firing rate when the contralateral sound level is more intense than the ipsilateral sound level (Ono et al., 2014; Xiong et al., 2013). These firing patterns might reflect the responses of the LSO neurons (see Discussion).

4. Discussion

4.1 Cell types

The present results demonstrated that over half of the neurons in the mouse IC respond an ITD in the envelope of a high-frequency sound. In the IC, the neurons are either GABAergic or glutamatergic, and both are sensitive to the envelope ITD. To the best of our knowledge, the neural sensitivity to the ITD in the envelope has not been shown in the auditory pathway of the mouse previously.

GABAergic and glutamatergic neurons in the IC have the opposite functions in the neural circuit: one is inhibitory and the other is excitatory. Our previous study showed that GABAergic and glutamatergic neurons shared similar response properties to pure tones (Ono et al., 2017). However, it also showed that the glutamatergic neurons follow the raised sine AM sound better than GABAergic neurons. In line with this observation, more glutamatergic neurons than GABAergic neurons tend to show synchronization to the beat frequency in the raised sine envelope (Fig. 2B), and this may be due to a subpopulation of glutamatergic neurons with high vector strength to envelope modulations (Fig. 2D). Strong synchronization to modulations is necessary to preserve the temporal information ascending in the auditory pathway; and glutamatergic neurons in the IC seem well-designed to do this. In contrast to glutamatergic neurons, GABAergic neurons may not contribute as much to transmission of the ITD information. However, the GABAergic neurons may contribute the sharpening of the ITD sensitivity of the postsynaptic neurons. In the medial superior olive (MSO) and nucleus laminaris (NL), avian analogue of MSO (Carr et al., 2016; Grothe et al., 2014; Lipovsek et al., 2018), ITD curves were suggested to be sharpen by inhibitory inputs (Brand et al., 2002; Funabiki et al., 1998; Nishino et al., 2008; Pecka et al., 2008; Yamada et al., 2013). Even though the inhibition from the GABAergic IC neuron may not convey ITD information, it might increase the conductance in the postsynaptic neurons (Funabiki et al., 1998) and sharpen the ITD tuning via ‘iceberg effect’ (Isaacson et al., 2011).

The role of ITD-sensitive glutamatergic and GABAergic neurons in the IC may depend on their interaction in neurons postsynaptic to IC inputs, for example in the medial geniculate body (MGB). If ITD sensitive inhibition and excitation from IC converge on the same postsynaptic neuron, it would act to control the gain of the postsynaptic excitation in the MGB since both GABAergic and glutamatergic IC neurons have ITD tuning with similar properties. In contrast, if ITD-sensitive inhibition combines with ITD-insensitive excitation, or the reverse, it could either enhance or suppress responses to ITDs > 50 μ sec. Inhibiting

the response to large ITDs outside the physiological range at the MGB level might be useful in that it might suppress the response to echoes and distortions.

4.2 The origin of the ITD sensitivity

In present data, most neurons in the mouse IC had trough-type ITD sensitivity. The trough-type ITD sensitivity is suggested to be shaped by the coincidence of bilateral excitatory-inhibitory synaptic inputs (Batra et al., 1997b; Grothe et al., 2014; Joris et al., 1995). Most IC neurons in mice have excitatory and inhibitory synaptic inputs driven by both ears (Ono et al., 2014; Ono et al., 2015). Thus, ITD sensitivity may be shaped in IC neurons by the interaction of bilateral monaural synaptic inputs. However, in order to obtain ITD sensitivity initially, it is necessary for the monaural contralateral and ipsilateral synaptic inputs to be highly phase-locked to the envelope. Our results suggest that the phase-locking of IC neurons to monaural sounds may be insufficient to explain the ITD sensitivity. The phase-locking to the monaural contralateral sound degraded in the IC neurons at higher modulation frequencies (Fig. 2C, D) despite the fact that some IC neurons had ITD-sensitivity to high-frequency modulation. Thus, it is unlikely that the ITD sensitivity to the high-frequency modulation is shaped within the IC itself.

It is more likely that ITD-sensitivity in the IC is inherited from the response properties of the neurons in the brainstem. The first binaural processing to produce ITD sensitivity occurs in the MSO and LSO. Anatomical observations show that the LSO is much larger than MSO in the mouse (Fischl et al., 2016; Ito et al., 2011). Thus, the ITD sensitivity of neurons in the mouse IC is most likely to be due to inputs from the LSO. Supporting this view, the previous studies in the cat and rabbit showed that the neurons in LSO could detect the envelope ITDs and had a trough-type ITD sensitivity (Batra et al., 1997c; Joris et al., 1995). Thus, LSO neurons in mice are likely to have trough-type ITD sensitivity because their LSO also has the bilateral excitatory-inhibitory synaptic inputs (Wu et al., 1991; Wu et al., 1992a; Wu et al., 1992b). Furthermore, recent studies of the LSO neurons in gerbil showed that they had fast membrane properties and synaptic properties similar to those in MSO and suitable for precise binaural timing (Beiderbeck et al., 2018; Franken et al., 2016). The LSO neurons in gerbil varied their firing probability when the ITD changed in the order of microseconds (Beiderbeck et al., 2018). This suggested that the LSO could detect ITD as well as ILD. So, even small mammals with an undeveloped MSO might be able to detect ITD via LSO neurons.

In the present data, the slope of most ITD curves in IC showed that the firing rate increased when the contralateral sound preceded the ipsilateral sound (Fig. 6). The IC neurons might inherit this response property from contralateral LSO neurons because the contralateral projections from LSO neurons are exclusively excitatory (Oliver et al., 1995). In contrast, the ipsilateral projections from LSO neurons are both excitatory and inhibitory (Oliver et al., 1995). Thus, the inputs from the ipsilateral LSO might be balanced and have a smaller effect on the IC neurons.

Besides LSO, the nuclei of the lateral lemniscus may contribute to binaural processing in the IC. The dorsal and ventral nuclei of the lateral lemniscus exclusively consist of inhibitory neurons; whereas, the intermediate nucleus of the lateral lemniscus is excitatory (Ito et al.,

2010). The previous studies have shown that the dorsal nucleus contains many binaural neurons sensitive to ITD as does a small medial region of the ventral nucleus (Batra et al., 1997a; Batra et al., 2002; Kelly et al., 1998; Pecka et al., 2010; Siveke et al., 2006). The intermediate nucleus has fewer binaural neurons (Kelly et al., 1998). Consequently, the dorsal nucleus in particular is likely to send binaurally modulated inhibition to the IC to modulate the ITD tuning in the IC (Pecka et al., 2010).

4.3 ITD sensitivity in the mouse

Most mammals with low-frequency hearing use the ongoing interaural phase differences in the fine structure of the stimuli in the two ears as a cue for sound localization. The mouse is unlikely to do this because of its exclusively high-frequency hearing and small head that suggests a narrow physiological ITD range (Allen et al., 2010; Radziwon et al., 2009). In line with this, mouse could not localize the sound when it consisted of only a low-frequency component in behavioral studies (Allen et al., <8 kHz. Heffner et al., <10 kHz) (Allen et al., 2010; Heffner, 2001). It is possible that ITD sensitivity to the neural envelope in IC is part of a neuronal circuit that is no longer used by mice for binaural hearing, similar to that discussed for the Mexican free-tailed bat (Grothe et al., 1998).

Only a few animal studies have investigated sound localization to the envelopes of high-frequency sounds. There are small animals like the Jamaican fruit bat and common vampire bat, similar in size to mice, that can use the envelope ITD as a sound localization cue (Heffner et al., 2001; Heffner et al., 2015), although other species of bats could not localize an AM tone with a low-frequency carrier (Heffner et al., 2010a; Heffner et al., 2010b). In laboratory rats, sound localization performance may be enhanced by amplitude modulation (Wesolek et al., 2010). Furthermore, a recent study showed that the rats could detect the ITD in the envelope (Li et al., 2019). In contrast, it is well established that human subjects can detect the ITD in high-frequency amplitude modulated tones (Bernstein et al., 1994; Bernstein et al., 2002; Bernstein et al., 2008; Bernstein et al., 2012; Klein-Hennig et al., 2011; McFadden et al., 1976; Nuetzel et al., 1976). This is despite the relatively limited high-frequency hearing in humans.

How may mice use the information from the envelope ITD? The small head size of a mouse may limit the “physiological” range of ITD to ± 50 μ sec, and the trough of the ITD curve covers most of that range. Nevertheless, mice might experience longer ITDs in a natural environment with sound reflections and echoes, and these could drive the LSO neurons. LSO activity would provide excitatory ITD-sensitive inputs to the contralateral IC. In contrast, LSO activity may provide ITD-sensitive inhibitory inputs to the ipsilateral IC. In either case, the LSO inputs could cause either glutamatergic or GABAergic neurons in IC to be sensitive to ITDs > 50 μ sec.

It is possible that the both ILD and ITD cooperate to enhance the firing rate when a sound source is positioned in contralateral side. In that scenario, the sound arrives earlier and stronger to the contralateral ear than to the ipsilateral ear. Most IC neurons have an ILD curve with increased firing rate when the contralateral sound level is higher than ipsilateral level (Ono et al., 2014). The present data shows that the most IC neurons increased their firing when the contralateral sound preceded the ipsilateral sound. However, the change in

firing rate within physiological ITD range was very small in most neurons (Fig. 6). So, the ITD may have minimal effects on the firing rate of the IC neurons. This is consistent with previous psychophysics observations that a large envelope ITD was necessary to produce a shift of the intracranial acoustic image away from the midline (Bernstein et al., 1985).

4.4 The effect of the envelope shape.

In the natural environment, both the carrier and the envelope of a modulated sound may have an ITD. Here, we used a binaural beat AM stimulus in which only the envelope had a binaural phase difference, but there was no phase difference in the carrier. A noise stimulus has an envelope as well as low frequency components where phase locking may occur and both may contribute to ITD sensitivity. However, in the present study, the mouse is unlikely to be sensitive to the low frequencies where phase locking may occur. So, only the envelope contributes to ITD sensitivity.

Human psychophysical studies have shown that the envelope ITD sensitivity is dependent on the temporal pattern of the waveform. The attack duration and the pause duration prior to the attack in the envelope are critical components that determine the envelope ITD sensitivity (Dietz et al., 2016; Klein-Hennig et al., 2011). In accordance with this, a listener's threshold ITD is lower for the raised sine AM sound than for a SAM (Bernstein et al., 2009). It is unclear what neuronal mechanism underlies this sensitivity to the envelope shape. However, it is very likely that the shape of the envelope affects the ITD sensitivity of the neurons in the auditory pathway. Our present data showed that the ITD sensitivity of the IC neurons was strongly dependent on the modulation frequency and the raised sine of the envelope (Fig. 2B). The ITD slope was slightly enhanced by raising the envelope (Fig. 6), and this might be relevant to the enhancement of ITD sensitivity. Furthermore, it is possible that even more neurons in the mouse IC might turn out to be sensitive to envelope ITD if they are tested with more diverse sound stimuli than those used in the present study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Drs. Shigeyuki Kuwada and Duck Kim for helpful advice on the data analysis. This work was supported by grants from NIH (grant number R01 DC000189 and R21 DC013822), UConn Health center (HCRAC grant 401139UCHC), JSPS KAKENHI (Grant Number JP16K11200, JP19K09918, JP19H04212 and JP17H02223), Kanazawa Medical University (S2016-8) and the Watanabe Foundation.

Abbreviations

IC	inferior colliculus
ICC	central nucleus of IC
ITD	interaural time difference
IPD	interaural phase difference

ILD	interaural level difference
MSO	medial superior olivary nucleus
LSO	lateral superior olivary nucleus
SOC	superior olivary complex
AM	amplitude modulation
SAM	sinusoidal amplitude modulation
ABR	auditory brainstem response
CF	characteristic frequency
BF	best frequency
rMTF	rate modulation transfer function
CP	characteristic phase
CD	characteristic delay
VS	vector strength
PFA	paraformaldehyde
SD	standard deviation
MAV	mean of abstract values

LITERATURE CITED

- Allen PD, Ison JR 2010 Sensitivity of the mouse to changes in azimuthal sound location: angular separation, spectral composition, and sound level. *Behavioral neuroscience* 124, 265–77. [PubMed: 20364886]
- Batra R, Fitzpatrick DC 1997a Neurons sensitive to interaural temporal disparities in the medial part of the ventral nucleus of the lateral lemniscus. *J Neurophysiol* 78, 511–5. [PubMed: 9242298]
- Batra R, Fitzpatrick DC 2002 Monaural and binaural processing in the ventral nucleus of the lateral lemniscus: a major source of inhibition to the inferior colliculus. *Hear Res* 168, 90–7. [PubMed: 12117512]
- Batra R, Kuwada S, Stanford TR 1989 Temporal coding of envelopes and their interaural delays in the inferior colliculus of the unanesthetized rabbit. *J Neurophysiol* 61, 257–68. [PubMed: 2918354]
- Batra R, Kuwada S, Stanford TR 1993 High-frequency neurons in the inferior colliculus that are sensitive to interaural delays of amplitude-modulated tones: evidence for dual binaural influences. *J Neurophysiol* 70, 64–80. [PubMed: 8395589]
- Batra R, Kuwada S, Fitzpatrick DC 1997b Sensitivity to interaural temporal disparities of low- and high-frequency neurons in the superior olivary complex. II. Coincidence detection. *J Neurophysiol* 78, 1237–47. [PubMed: 9310415]
- Batra R, Kuwada S, Fitzpatrick DC 1997c Sensitivity to interaural temporal disparities of low- and high-frequency neurons in the superior olivary complex. I. Heterogeneity of responses. *J Neurophysiol* 78, 1222–36. [PubMed: 9310414]

- Beiderbeck B, Myoga MH, Muller NIC, Callan AR, Friauf E, Grothe B, Pecka M 2018 Precisely timed inhibition facilitates action potential firing for spatial coding in the auditory brainstem. *Nature communications* 9, 1771.
- Bernstein LR, Trahiotis C 1985 Lateralization of low-frequency, complex waveforms: the use of envelope-based temporal disparities. *The Journal of the Acoustical Society of America* 77, 1868–80. [PubMed: 3998297]
- Bernstein LR, Trahiotis C 1994 Detection of interaural delay in high-frequency sinusoidally amplitude-modulated tones, two-tone complexes, and bands of noise. *The Journal of the Acoustical Society of America* 95, 3561–7. [PubMed: 8046145]
- Bernstein LR, Trahiotis C 2002 Enhancing sensitivity to interaural delays at high frequencies by using "transposed stimuli". *The Journal of the Acoustical Society of America* 112, 1026–36. [PubMed: 12243151]
- Bernstein LR, Trahiotis C 2008 Discrimination of interaural temporal disparities conveyed by high-frequency sinusoidally amplitude-modulated tones and high-frequency transposed tones: effects of spectrally flanking noises. *The Journal of the Acoustical Society of America* 124, 3088–94. [PubMed: 19045794]
- Bernstein LR, Trahiotis C 2009 How sensitivity to ongoing interaural temporal disparities is affected by manipulations of temporal features of the envelopes of high-frequency stimuli. *The Journal of the Acoustical Society of America* 125, 3234–42. [PubMed: 19425666]
- Bernstein LR, Trahiotis C 2012 Lateralization produced by interaural temporal and intensive disparities of high-frequency, raised-sine stimuli: data and modeling. *The Journal of the Acoustical Society of America* 131, 409–15. [PubMed: 22280602]
- Brand A, Behrend O, Marquardt T, McAlpine D, Grothe B 2002 Precise inhibition is essential for microsecond interaural time difference coding. *Nature* 417, 543–7. [PubMed: 12037566]
- Carr CE, Christensen-Dalsgaard J 2016 Evolutionary trends in directional hearing. *Current opinion in neurobiology* 40, 111–117. [PubMed: 27448850]
- Choy Buentello D, Bishop DC, Oliver DL 2015 Differential distribution of GABA and glycine terminals in the inferior colliculus of rat and mouse. *J Comp Neurol* 523, 2683–97. [PubMed: 25976159]
- Dietz M, Marquardt T, Salminen NH, McAlpine D 2013 Emphasis of spatial cues in the temporal fine structure during the rising segments of amplitude-modulated sounds. *Proc Natl Acad Sci U S A* 110, 15151–6. [PubMed: 23980161]
- Dietz M, Wang L, Greenberg D, McAlpine D 2016 Sensitivity to Interaural Time Differences Conveyed in the Stimulus Envelope: Estimating Inputs of Binaural Neurons Through the Temporal Analysis of Spike Trains. *J Assoc Res Otolaryngol* 17, 313–30. [PubMed: 27294694]
- Fischl MJ, Burger RM, Schmidt-Pauly M, Alexandrova O, Sinclair JL, Grothe B, Forsythe ID, Kopp-Scheinflug C 2016 Physiology and anatomy of neurons in the medial superior olive of the mouse. *J Neurophysiol* 116, 2676–2688. [PubMed: 27655966]
- Franken TP, Smith PH, Joris PX 2016 In vivo Whole-Cell Recordings Combined with Electron Microscopy Reveal Unexpected Morphological and Physiological Properties in the Lateral Nucleus of the Trapezoid Body in the Auditory Brainstem. *Front Neural Circuits* 10, 69. [PubMed: 27605909]
- Funabiki K, Koyano K, Ohmori H 1998 The role of GABAergic inputs for coincidence detection in the neurones of nucleus laminaris of the chick. *J Physiol* 508 (Pt 3), 851–69. [PubMed: 9518738]
- Grothe B, Park TJ 1998 Sensitivity to interaural time differences in the medial superior olive of a small mammal, the Mexican free-tailed bat. *J Neurosci* 18, 6608–22. [PubMed: 9698347]
- Grothe B, Pecka M 2014 The natural history of sound localization in mammals—a story of neuronal inhibition. *Front Neural Circuits* 8, 116. [PubMed: 25324726]
- Heffner RS, Koay G, Heffner HE 2001 Sound localization in a new-world frugivorous bat, *Artibeus jamaicensis*: acuity, use of binaural cues, and relationship to vision. *The Journal of the Acoustical Society of America* 109, 412–21. [PubMed: 11206172]
- Heffner RS, Koay G, Heffner HE 2010a Use of binaural cues for sound localization in two species of Phyllostomidae: the Greater spear-nosed bat (*Phyllostomus hastatus*) and the Short-tailed fruit bat (*Carollia perspicillata*). *Journal of comparative psychology* 124, 447–54. [PubMed: 21090890]

- Heffner RS, Koay G, Heffner HE 2010b Use of binaural cues for sound localization in large and small non-echolocating bats: *Eidolon helvum* and *Cynopterus brachyotis*. *The Journal of the Acoustical Society of America* 127, 3837–45. [PubMed: 20550282]
- Heffner RS, Koay G, Heffner HE 2015 Sound localization in common vampire bats: acuity and use of the binaural time cue by a small mammal. *The Journal of the Acoustical Society of America* 137, 42–52. [PubMed: 25618037]
- Heffner RSK, Heffner G, H. E. 2001 Focus: Sound-Localization Acuity Changes with Age in C57BL/6J mice. In: Willott JF., (Ed.), *Handbook of Mouse Auditory Research: From Behaviour to Molecular Biology*. CRC Press pp. 31–35.
- Isaacson JS, Scanziani M 2011 How inhibition shapes cortical activity. *Neuron* 72, 231–43. [PubMed: 22017986]
- Ito T, Oliver DL 2010 Origins of Glutamatergic Terminals in the Inferior Colliculus Identified by Retrograde Transport and Expression of VGLUT1 and VGLUT2 Genes. *Front Neuroanat* 4, 135. [PubMed: 21048892]
- Ito T, Bishop DC, Oliver DL 2011 Expression of glutamate and inhibitory amino acid vesicular transporters in the rodent auditory brainstem. *The Journal of Comparative Neurology* 519, 316–340. [PubMed: 21165977]
- Joris PX, Yin TC 1995 Envelope coding in the lateral superior olive. I. Sensitivity to interaural time differences. *J Neurophysiol* 73, 1043–62. [PubMed: 7608754]
- Joris PX, Schreiner CE, Rees A 2004 Neural processing of amplitude-modulated sounds. *Physiological reviews* 84, 541–77. [PubMed: 15044682]
- Keating P, Nodal FR, Gananandan K, Schulz AL, King AJ 2013 Behavioral sensitivity to broadband binaural localization cues in the ferret. *J Assoc Res Otolaryngol* 14, 561–72. [PubMed: 23615803]
- Kelly JB, Buckthorpe AD, Kidd SA 1998 Monaural and binaural response properties of single neurons in the rat's dorsal nucleus of the lateral lemniscus. *Hear Res* 122, 25–40. [PubMed: 9714572]
- Klein-Hennig M, Dietz M, Hohmann V, Ewert SD 2011 The influence of different segments of the ongoing envelope on sensitivity to interaural time delays. *The Journal of the Acoustical Society of America* 129, 3856–72. [PubMed: 21682409]
- Kuwada S, Stanford TR, Batra R 1987 Interaural phase-sensitive units in the inferior colliculus of the unanesthetized rabbit: effects of changing frequency. *J Neurophysiol* 57, 1338–60. [PubMed: 3585471]
- Li K, Chan CHK, Rajendran VG, Meng Q, Rosskoth-Kuhl N, Schnupp JWH 2019 Microsecond sensitivity to envelope interaural time differences in rats. *The Journal of the Acoustical Society of America* 145, EL341. [PubMed: 31153346]
- Lipovsek M, Wingate RJ 2018 Conserved and divergent development of brainstem vestibular and auditory nuclei. *eLife* 7.
- McAlpine D, Jiang D, Palmer AR 2001 A neural code for low-frequency sound localization in mammals. *Nat Neurosci* 4, 396–401. [PubMed: 11276230]
- McFadden D, Pasanen EG 1976 Lateralization of high frequencies based on interaural time differences. *The Journal of the Acoustical Society of America* 59, 634–9. [PubMed: 1254790]
- Nishino E, Yamada R, Kuba H, Hioki H, Furuta T, Kaneko T, Ohmori H 2008 Sound-intensity-dependent compensation for the small interaural time difference cue for sound source localization. *J Neurosci* 28, 7153–64. [PubMed: 18614685]
- Nuetzel JM, Hafter ER 1976 Lateralization of complex waveforms: effects of fine structure, amplitude, and duration. *The Journal of the Acoustical Society of America* 60, 1339–46. [PubMed: 1010886]
- Oliver DL, Beckius GE, Shneiderman A 1995 Axonal projections from the lateral and medial superior olive to the inferior colliculus of the cat: a study using electron microscopic autoradiography. *J Comp Neurol* 360, 17–32. [PubMed: 7499562]
- Ono M, Oliver DL 2014 The balance of excitatory and inhibitory synaptic inputs for coding sound location. *J Neurosci* 34, 3779–92. [PubMed: 24599475]
- Ono M, Ito T 2015 Functional organization of the mammalian auditory midbrain. *The journal of physiological sciences : JPS* 65, 499–506. [PubMed: 26362672]

- Ono M, Bishop DC, Oliver DL 2016 Long-Lasting Sound-Evoked Afterdischarge in the Auditory Midbrain. *Scientific reports* 6, 20757. [PubMed: 26867811]
- Ono M, Bishop DC, Oliver DL 2017 Identified GABAergic and Glutamatergic Neurons in the Mouse Inferior Colliculus Share Similar Response Properties. *J Neurosci* 37, 8952–8964. [PubMed: 28842411]
- Park TJ, Grothe B, Pollak GD, Schuller G, Koch U 1996 Neural delays shape selectivity to interaural intensity differences in the lateral superior olive. *J Neurosci* 16, 6554–66. [PubMed: 8815932]
- Pecka M, Brand A, Behrend O, Grothe B 2008 Interaural time difference processing in the mammalian medial superior olive: the role of glycinergic inhibition. *J Neurosci* 28, 6914–25. [PubMed: 18596166]
- Pecka M, Siveke I, Grothe B, Lesica NA 2010 Enhancement of ITD coding within the initial stages of the auditory pathway. *J Neurophysiol* 103, 38–46. [PubMed: 19846624]
- Radziwon KE, June KM, Stolzberg DJ, Xu-Friedman MA, Salvi RJ, Dent ML 2009 Behaviorally measured audiograms and gap detection thresholds in CBA/CAJ mice. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* 195, 961–9.
- Rose JE, Brugge JF, Anderson DJ, Hind JE 1967 Phase-locked response to low-frequency tones in single auditory nerve fibers of the squirrel monkey. *J Neurophysiol* 30, 769–93. [PubMed: 4962851]
- Siveke I, Pecka M, Seidl AH, Baudoux S, Grothe B 2006 Binaural response properties of low-frequency neurons in the gerbil dorsal nucleus of the lateral lemniscus. *J Neurophysiol* 96, 1425–40. [PubMed: 16571733]
- Wesolek CM, Koay G, Heffner RS, Heffner HE 2010 Laboratory rats (*Rattus norvegicus*) do not use binaural phase differences to localize sound. *Hear Res* 265, 54–62. [PubMed: 20184949]
- Wu SH, Kelly JB 1991 Physiological properties of neurons in the mouse superior olive: membrane characteristics and postsynaptic responses studied in vitro. *J Neurophysiol* 65, 230–46. [PubMed: 2016640]
- Wu SH, Kelly JB 1992a Binaural interaction in the lateral superior olive: time difference sensitivity studied in mouse brain slice. *J Neurophysiol* 68, 1151–9. [PubMed: 1432074]
- Wu SH, Kelly JB 1992b Synaptic pharmacology of the superior olivary complex studied in mouse brain slice. *J Neurosci* 12, 3084–97. [PubMed: 1494947]
- Xiong XR, Liang F, Li H, Mesik L, Zhang KK, Polley DB, Tao HW, Xiao Z, Zhang LI 2013 Interaural level difference-dependent gain control and synaptic scaling underlying binaural computation. *Neuron* 79, 738–53. [PubMed: 23972599]
- Yamada R, Okuda H, Kuba H, Nishino E, Ishii TM, Ohmori H 2013 The cooperation of sustained and phasic inhibitions increases the contrast of ITD-tuning in low-frequency neurons of the chick nucleus laminaris. *J Neurosci* 33, 3927–38. [PubMed: 23447603]
- Yin TC, Kuwada S 1983a Binaural interaction in low-frequency neurons in inferior colliculus of the cat. II. Effects of changing rate and direction of interaural phase. *J Neurophysiol* 50, 1000–19. [PubMed: 6631458]
- Yin TC, Kuwada S 1983b Binaural interaction in low-frequency neurons in inferior colliculus of the cat. III. Effects of changing frequency. *J Neurophysiol* 50, 1020–42. [PubMed: 6631459]
- Yin TC, Chan JC 1990 Interaural time sensitivity in medial superior olive of cat. *J Neurophysiol* 64, 465–88. [PubMed: 2213127]
- Zhao S, Ting JT, Atallah HE, Qiu L, Tan J, Gloss B, Augustine GJ, Deisseroth K, Luo M, Graybiel AM, Feng G 2011 Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. *Nature methods* 8, 745–52. [PubMed: 21985008]

- Over half of neurons in the mouse IC were sensitive to ITD of the sound envelope.
- Both GABAergic and glutamatergic neurons responded to ITD of the sound envelope.
- The envelope ITD curves of most IC neurons in the mouse were aligned at troughs.
- The ITD-evoked response was stronger when the contralateral sound preceded.

Fig. 1.

The analysis of the neuronal responses to binaural beat AM sound. (A) An example of the raster plot of the response to binaural beat AM sound. The responses were recorded from a glutamatergic neuron. (B) The period histograms of the response in A. Each panel is the histogram of the response to different modulation frequency. Note that the left panel (32 Hz) has a different scale of y axis from other panels (64 – 512 Hz). (C) The ITD curves of the response in A. Each curve corresponds to different modulation frequency. The left and the right panel show the same curves in the different scale in x-axis. In the left panel, the broader curve in x axis corresponds to the response to the lower modulation frequency. A thick black curve in the right panel indicates a composite ITD curve. (D) The ITD curves of a GABAergic neuron.

Fig. 2.

Both GABAergic and glutamatergic neurons synchronized to binaural beat AM sound. (A) The percentage of the beat-sensitive neurons to binaural SAM (left) and raised sine (right) sounds. (B) The percentage of synchronized neurons to binaural beat frequency in the envelopes of SAM (left) and raised sine (right) with different modulation frequencies. The numbers of tested neurons were as follows: For 32, 64, 128, 256, and 512 Hz, $n = 16, 17, 17, 17,$ and 17 (GABA-SAM). $n = 26, 26, 25, 25,$ and 25 (GLU-SAM). $n = 10, 12, 12, 12,$ and 12 (GABA-raised). $n = 19, 21, 20, 21, 21,$ and 21 (GLU-raised). (C, D) Vector strength to the binaural beat VS_{beat} plotted against vector strength to the contralateral stimulus VS_{contra} . (C) SAM. (D) Raised sine. The upper and bottom plots showed the individual and averaged data, respectively.

Fig. 3. rMTFs of the IC neurons to binaural beat AM sound. (A-E) GABAergic neurons. (F-J) glutamatergic neurons. (A, F) Low rate. (B, G) Low pass. (C, H) Band reject. (D, I) High pass. (E, J) Others. Others contains the band pass, all pass and multipeak. The left and right panels are the rMTF to SAM and raised sine, respectively. Different colors and symbols represent the rMTFs from different neurons. The same color and symbol in the left and right panels represent the rMTF from the same neuron. The filled and unfilled symbols indicate the synchronized and unsynchronized responses to envelope beat, respectively.

Fig.4.

Boxplots of the firing rate in response to different modulation frequencies. (A) SAM (B) Raised sine. The box represents 25th, 50th, 75th percentiles. Square in the box represents the mean value. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. Tukey HSD test.

Fig.5.

Most IC neurons were trough-type. (A-D) The samples of ITD curves. Different colors indicate the responses to different modulation frequencies as in Fig. 1C and D. (A, B) Trough type. (C) Peak type. (D) Not aligned. The cell and envelope types are as follows: (A) Glutamatergic. SAM. (B) GABAergic. SAM. (C) glutamatergic. Raised sine. (D) Glutamatergic. SAM. (E-H) Phase plot analysis of ITD curves. (E, F) The phase plots of the responses from glutamatergic neurons (E) and GABAergic neurons (F). Mean interaural phase was plotted against the modulation frequency and fitted with linear regression. (E) The plots from left correspond to the responses shown in Fig. 1C, 5A, 5C, and 5D. (F) The plots from left correspond to the responses shown in Fig. 1D and 5B. (G) Dot plots of the characteristic phase. (H) Characteristic delay was plotted against mean trough (left panel) and peak (right panel) time.

Fig. 6.

The slope of ITD curves within the physiological ITD range of mice. (A, B) The normalized ITD curves were presented within ± 0.2 ms. Left panel, GABAergic. Right panel, glutamatergic. The gray box indicates the physiological ITD range of mice ($\pm 50 \mu s$). (A) SAM. (B) Raised sine. (C, D) The histograms of the slope of ITD curve. Left panel, GABAergic. Right panel, glutamatergic. (C) SAM (D) Raised sine.

Table 1

The distribution of the envelope ITD-sensitive neurons

	GABA-SAM	GABA-raised	Glu-SAM	Glu-raised
Area 1	n = 3 (3)	n = 1 (2)	n = 6 (8)	n = 7 (7)
Border	n = 1 (1)	n = 1 (1)	n = 3 (3)	n = 3 (3)
Area 2	n = 2 (4)	n = 1 (4)	n = 1 (2)	n = 1 (1)
LC	n = 1 (3)	n = 1 (1)	n = 3 (3)	n = 2 (2)
Ventral	n = 1 (1)	n = 1 (1)	n = 1 (3)	n = 1 (3)