INTERAURAL ONSET TIME DIFFERENCES IN THE BAT

SENSITIVITY TO INTERAURAL ONSET TIME DIFFERENCES OF HIGH FREQUENCY STIMULI IN THE INFERIOR COLLICULUS OF *EPTESICUS FUSCUS*

By

ZEESHAN HAQQEE

Honours Bachelor of Science, McMaster University, 2015

A Thesis Submitted in Partial Fulfillment

of the Requirements of the Degree of

Master of Science

in the Department of Psychology, Neuroscience & Behaviour

Faculty of Science

McMaster University MASTER OF SCIENCE (2018) Hamilton, Ontario

Department of Psychology, Neuroscience & Behaviour

TITLE: Sensitivity to interaural onset time differences of high frequency stimuli in the inferior colliculus of *Eptesicus fuscus.*

AUTHOR: Zeeshan Haqqee, BSc.

SUPERVISOR: Dr. Paul A. Faure

NUMBER OF PAGES: ix, 55

Abstract

Many neurons in the auditory midbrain are tuned to binaural cues. Two prominent binaural cues are the interaural intensity difference (IID) and the interaural time difference (ITD). The ITD cue can further be classified as either an ongoing ITD, which compares the phase difference in the waveform of low frequency stimuli present at either ear, or an onset ITD, which compares the onset time of arrival of two stimuli at either ear. Little research has been done on the sensitivity of single neurons to onset ITDs in the auditory system, particularly in bats. The current study examines the response properties of neurons in the inferior colliculus (IC) of the big brown bat, *Eptesicus fuscus*, to onset ITDs in response to high frequency pure tones. Measures of neurons' dynamic response—the segment of the ITD function exhibiting the highest rate of change in activity—revealed an average change of 36% of its maximum response within the estimated behaviorally relevant range of ITDs. Time-intensity trading describes the ability of the brain to compensate the binaural time cue (ITD) cue for the binaural intensity cue (IID) and can be measured as the horizontal shift of an ITD function at various IIDs. Across all IC neurons, an average time-intensity trading ratio of 30 μs/dB was calculated to measure the sensitivity of IC neurons' ITD response to changing IIDs. Minimum and maximum ITD responses were found to be clustered within a narrow range of ITDs. The average peak ITD response occurred at 268 μs and is consistent with findings in other mammals. All results in ITD tuning, time-intensity trading, and response maximum were invariant to stimulus frequency, confirming that IC neurons responded to

onset ITDs and not ongoing ITDs. These results suggest the potential for high frequency onset cues to assist in the azimuthal localization of sound in echolocating bats.

Acknowledgements

I would like to thank my supervisor, Dr. Paul A. Faure, for guiding and supporting me through my graduate experience here at McMaster University and for allowing me the chance to work with one of the most fascinating animal species on the planet.

I would also like to thank all the members of the bat lab who have helped me along my journey as a Masters student, as well as the Central Animal Facility staff for taking care of our bats. I would like to extend a special thank you to Roberto Valdizon for the countless hours he has dedicated to helping me learn surgical and recording techniques and for teaching me the invaluable coding skills necessary to complete this project. I am grateful for the opportunity to learn neuroscience research techniques in the wonderfully cohesive environment provided by the McMaster Batlab.

Finally, I would like to thank my parents, Shamsa and Adnan Haqqee, for always supporting me in my ambitions to succeed in academia.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

BD – best duration

BEF – best excitatory frequency

Contra – contralateral

Dur – Stimulus duration

EE – excitatory contralaterally, excitatory ipsilaterally

EI – excitatory contralaterally, inhibitory ipsilaterally

EO – excitatory ipsilaterally, no input ipsilaterally

IC – inferior colliculus

IID – interaural intensity difference

Ipsi – ipsilateral

ITD – interaural time difference

LSO – lateral superior olive

MSO – medial superior olive

SE – standard error

SD – standard deviation

1. Introduction

The duplex theory of sound localization, first described by Rayleigh (1907), relates binaural cue sensitivity to stimulus frequency. The theory states that low frequency sounds are processed by comparing the interaural time difference (ITD) between the two ears, whereas high frequency sounds are localized by comparing the interaural intensity difference (IID) between the two ears. The ITD cue occurs due to differences in arrival time of a sound stimulus at either ear. This can be measured as the difference in the onset time of a stimulus arriving at each ear, as well as the ongoing phase difference in the carrier frequency of the stimulus waveform or its envelope. Studies in human psychophysics demonstrate that ongoing phase differences appear to be a more powerful cue than transient onset differences in azimuthal localization (Buell et al., 1991; Tobias and Shubert, 1959). However, at increasingly higher frequencies, the stimulus wavelength decreases and consequently shortens the period over which phase comparisons can reliably be made between two stimuli. Hence, the ongoing phase difference between the two ears becomes more ambiguous at higher stimulus frequencies, leading to the convention that high frequency hearing animals with small interaural distances should be incapable of processing ITDs. Despite this convention, many behavioral studies have shown several exceptions to this convention, where gerbils, least weasels, and certain species of bats have displayed sensitivity to both binaural cues, despite having head sizes comparable to species that only use the binaural intensity cues (for review, see Heffner and Heffner, 2016).

Bats present a particularly interesting case because the superior olivary nucleus that is believed to process ITDs, the medial superior olive (MSO), varies significantly in its neural architecture between different bat species (Grothe et al., 2001; for review, see Covey 2005). Many bat species also have a larger MSO relative to their head size compared to other mammalian species, although this increase has been attributed to an increase in monaural neural input only (for review, see Grothe & Park, 2000). The MSO of at least one bat species, the free-tailed bat, has been shown to respond to ongoing ITDs in the millisecond range, similar to that of the MSO in cats (Grothe and Park, 1998). However, this response range was far outside of the microsecond range of behaviorally relevant ITDs for the bat, which is the range of ITDs it would encounter while localizing sound in a natural setting. Another study reported sensitivity to onset ITDs in some neurons within the MSO of the molossus bat that were within the behaviorally relevant range of the animal (Harnischfeger et al., 1985). Research is thus inconclusive as to whether bats use their MSO to process ITDs, as it appears better suited in processing the temporal structure of sound to assist in echolocation (Grothe et al., 2001).

Despite the anomalies in the neurophysiology of the MSO between bats, some species have demonstrated the ability to localize low frequency sounds; indicating that they are able to process ongoing ITDs (Heffner et al., 2015, Heffner et al., 1999). This includes the Jamaican fruit bat, which despite not possessing an identifiable MSO (Zook & Casseday, 1980) is still capable of localizing low frequency pure tones (Heffner at al., 2001).

One possibility for these observations is that other brain areas besides the MSO may be responsible for processing ITDs in bats. In cats, the lateral superior olive (LSO)–a superior olivary nucleus responsible for coding IIDs–consists of neurons with demonstrated sensitivity to ongoing ITDs in response to low-frequency pure tones (Tollin & Yin, 2005) as well as to amplitude modulated envelopes of high frequency tones (Joris et al., 1995). Some evidence exists to propose the LSO as a likely candidate for ITD coding in bats (Grothe and Park, 1995); however, this only appears to be true at contralateral-leading ITDs in the range of hundreds of microseconds, far outside of the bat's behaviorally relevant range of ITDs, meaning that the LSO would not be able to process ITDs in natural conditions. It should also be noted that the LSO appears to be similar across all mammals in both structure and function, including in bats (Covey and Casseday, 1995), making it difficult to explain how it could allow for localization of ITDs in some bat species but not in others.

Further along the ascending pathway from the superior olivary complex is the inferior colliculus (IC) where inputs from the MSO, LSO, and other brainstem nuclei converge (Schofield, 2005). Evidence suggests that the IC recalculates certain binaural cues, such as IIDs (Pollak, 2012; Li et al., 2010). In numerous bat species, it also demonstrates some level of sensitivity to ongoing ITDs in the amplitude-modulated envelope of the stimulus (Fuzessery, 1997) as well as to transient onset ITDs (Sayegh et al., 2014; Pollak, 1988; Harnischfeger et al., 1985) Nevertheless, a systematic evaluation of ITD sensitivity in the IC of bats is lacking, particularly for transient onset ITDs.

In this study, I evaluated the ITD sensitivity of neurons in the IC of the big brown bat, *Eptesicus fuscus*. As most previous work has focused on the interaural phase difference cue, I focused here on the less studied transient onset delay cue, which has been shown to be necessary in the lateralization of auditory stimuli (Dietz et al., 2013; Perrott, 1969) and is independent to the response sensitivity of ongoing ITDs (Kuwada and Yin, 1983). A previous study demonstrated that neurons in the IC of *E. fuscus* display some form of ITD sensitivity (Sayegh et al., 2014), but did not perform an in-depth evaluation of the cells' dynamic response range and sensitivity to time-intensity trading. These measures provide a more thorough understanding of the degree to which neurons in the IC of bats are sensitive to ITDs, and measures of time-intensity trading ratios can be used to indicate relative sensitivities of IIDs and ITDs in shaping the binaural response properties of IC neurons (Pollak, 1988).

2. Materials and methods

Fourteen big brown bats (*Eptesicus fuscus)* of both sexes (6 males, 8 females) were used in this study. All procedures were approved by the McMaster University Animal Research Ethics Board and were in accordance with the guidelines for the care and use of experimental animals published by the Canadian Council on Animal Care.

2.1 Surgical procedures

To prepare for electrophysiological recordings, each animal underwent a posting surgery to attach a stainless-steel post to the dorsal surface of the skull, rostral to the IC. After subcutaneous administration of buprenorphine (Temgesic; ~0.03 mL SQ; 0.045

mg/kg), the bat was placed in a small anesthetic induction chamber (12x10x10cm; lxwxh) that continuously circulated a 1-5% v/v mixture of isoflurane and oxygen, (flow rate = 1L/min). Breathing rate was visually monitored until the bat appeared anesthetized. The bat was then placed in a foam-lined body support for surgery. The bat's teeth were positioned in a custom bite bar and a gas mask was fitted over the nose to ensure a continuous, controlled administration of isoflurane. Once the head was secured in the bite bar, the hair on its scalp was shaved and the scalp was disinfected with a povidone-iodine surgical scrub (Betadine®). A local anesthetic (~0.2mL of Bupivacaine (5mg/mL)) was applied before making a midline incision on the scalp. The temporal muscles were then reflected, and the dorsal surface of the skull was exposed. After gently scraping the surface clean with a bone scraper, the surface of the skull was then wiped with 70% ethanol:water v/v. Once dried, a headpost was glued to the skull overlying the cortex with cyanoacrylate superglue (Henkel Loctite Corporation) cured with liquid acrylic hardener (Zipkicker; Pacer Technology). A chloride silver wire attached to the headpost was then placed under the temporal muscles to serve as a reference electrode. Once the posting surgery was complete, the wound opening was covered with a piece of Gelfoam and coated with Polysporin to prevent infection. The bat was then allowed 1-3 days to recover from surgery before commencing recording. During this time, and between recording sessions, the bat was housed in its own stainless-steel cage (¼-in. mesh) in a temperature and humidity-controlled holding room and was supplied with food and water *ad libidum.*

2.2 Acoustic stimulation

Custom software was used to generate sound stimuli, as well as to display spike-times as dot raster displays. Pure tones were generated digitally from a two-channel array processor (TDT Apos II; 357 kHz sampling rate) optically interfaced to two digital-to-analog (D/A) converters (TDT DA3-2) whose individual outputs were fed to a low-pass antialiasing filter (TDT FT6-2; f_c =120 kHz), a programmable attenuator (TDT PA5), and two signal mixers (TDT SM5) with equal weighting. The output of each speaker was measured with a B&K type 4138 ⅛-in. condenser microphone (90° incidence; grid off) connected to a measuring amplifier (B&K type 2606) and a band-pass filter (B&K type 4231) and was expressed in decibels sound pressure level (dB re 20 µPa) equivalent to the peak amplitude of continuous tones of the same frequency. All pure tones had rise/fall times of 0.4 ms, shaped with a cosine squared function and presented at a rate of 3 Hz. Each loudspeaker was placed ~1 mm in front of the external auditory meatus. The loudspeaker transfer function was flat ±6 dB from 28 to 118 kHz and there was at least 30 dB attenuation at the ear opposite the source (Ehrlich et al., 1997).

2.3 Single-unit electrophysiology:

Electrophysiological recordings were performed inside a double-walled, sound attenuating booth with electrical shielding (Industrial Acoustics Co., Inc). Prior to recording, the bat was administered a subcutaneous injection of neuroleptic (~0.3 mL midazolam + fentanyl citrate: 1:1 v/v mixture of 0.25mg/mL of fentanyl citrate and 1.0 mg/mL midazolam). About 10 minutes post-injection, the bat was placed into a custom

foam-lined body restraint, which was suspended by springs within a stereotaxic frame (ASI Instruments). The head post was then secured into a stainless-steel rod attached to a micro manipulator (ASI Instruments). The entire stereotaxic setup rested atop an air vibration table (TMC Micro-G).

Once the Gelfoam was removed, a small craniotomy was performed with a scalpel to expose the IC for recording. Thin wall borosilicate glass microelectrodes (outside diameter = 1.2 mm; A-M Systems) filled with 1.5M NaCl were used to perform single unit extracellular recordings. During recording, electrode resistances typically ranged between 15-25 MΩ. After manually positioning the electrode above the craniotomy, a stepping hydraulic micropositioner (Kopf Model 2650) was used to lower the electrode into the IC. Action potentials were recorded with a neuroprobe amplifier (Model 1600; A-M Systems) whose 10x output was bandpass filtered and further amplified (500-1000x) by a Tucker Davis Technologies spike conditioner (TDT-PC1; lowpass fc = 7kHz; highpass fc = 300 Hz). Action potential spike times were logged by a computer passing the PC1 output to a spike discriminator (TDT-SD1), followed by an event timer (TDT-ET1) synchronized to a timing generator (TDT-TG6).

Recordings lasted between 6-8 hours, with each bat used in up to eight recording sessions. Recordings were terminated if the bat showed any signs of discomfort during any point of the procedure.

2.4 Data acquisition

Single units were isolated using two pure tones of 1 and 4 ms (Inter-stimulus interval = 110 ms) as search stimuli, presented monaurally to the ear contralateral to the IC being recorded. Search stimuli were also varied in frequency at 1 kHz intervals between 15 to 75 kHz, depending on the electrode depth. Upon isolation of a unit, I varied the stimulus frequency (0.5 to 1-kHz resolution) and defined the best excitatory frequency (BEF) as the stimulus that evoked maximal spiking. I then varied stimulus duration (0.1-1 ms resolution, minimum duration = 1ms) and defined the best duration (BD) as the stimulus that evoked maximal spiking to BEF tones. If the neuron was not duration selective to any of the test durations between 1 and 25 ms, I selected the shortest duration that evoked robust spiking near to the maximum response. Both BEF and BD were determined with stimuli at +10 dB above threshold of response to the contralateral ear.

Upon determining the monaural, contralaterally-evoked response properties of a cell, I used various binaural stimulation paradigms to explore the cell's response to IIDs, ITDs, and time-intensity trading. Where applicable, a neuron's BEF was compared across ITD types and tuning characteristics to determine if the cell's response to these binaural cues was at all dependent on the stimulus frequency, which could indicate a level of sensitivity to the phase differences in the stimulus waveforms, rather than its onset difference.

A cell's IID sensitivity was tested by holding the intensity at the contralateral ear at +10 dB above threshold while randomly varying the intensity in the ipsilateral ear (3 dB increments), ranging between ± 15 dB and ±30 dB relative to the contralateral stimulus. Both ipsilateral and contralateral stimuli were presented at the cell's BEF and BD (or minimum duration), determined from monaural, contralateral stimulation. The ipsilateral ear was also tested monaurally with this same stimulus to determine if the neuron was excited by ipsilateral stimulation alone. By examining the cell's responses to IID and monaural ipsilateral stimulation, I was able to classify the binaural response profile of the neuron as EE (excitatory response from stimulation to either ear), EI (excitatory response from contralateral stimulation, inhibitory response from ipsilateral stimulation), or EO (excitatory response from contralateral stimulation, no response from ipsilateral stimulation). This classification strategy was adapted from Fuzessery and Pollak (1985). If the neuron did not show any IID sensitivity upon testing at various ipsilateral intensities, it was determined that the neuron was not sensitive to intensity differences and was classified as EO. If IID sensitivity was observed with no excitatory response from ipsilateral stimulation alone, the neuron was classified as EI. If both IID sensitivity and ipsilaterally evoked activity was observed, the neuron was classified as EE. Some neurons only showed IID sensitivity and/or ipsilaterally evoked responses at specific stimulus intensities and were assigned mixed classifications.

A cell's ITD sensitivity was tested by holding the intensity at both ears at +10 dB above threshold (re contra ear) while randomly varying the onset time of the ipsilateral stimulus. An ITD is calculated by subtracting the onset time of the stimulus at the

ipsilateral ear from the onset time of the stimulus at the contralateral ear. Positive values indicate by how much the contralateral ear stimulus led the ipsilateral ear stimulus, while negative values indicate by how much the ipsilateral ear stimulus led the contralateral ear stimulus. An example of the stimulation paradigm used to generate ITD functions is shown in Figure 1. All neurons were tested within a maximum ITD range of ±1,000 μs, relative to the onset of the contralateral stimulus, at a resolution of 50μs. A few neurons were also tested up to ±10,000 μs at a resolution of 300 μs.

The cell's ability to trade time for intensity was tested by repeating ITD testing at various IIDs. If the neuron did not show ITD sensitivity when tested with a binaural stimulus of equal intensity (IID = 0 dB), I then increased the intensity at the ipsilateral ear (3-6 dB increments) with the expectation that increased ipsilateral inhibition would affect the binaural response of the cell (Figure 4). If the neuron did not show ITD sensitivity upon testing at different ipsilateral intensities, it was determined to be non-selective to ITDs.

For time-intensity trading, IIDs were varied in increments of 3-6 dB up to a maximum IID range of ±21 dB. Note that a negative IID value for time-intensity trading results always indicates an increased intensity at the ipsilateral ear relative to the contralateral ear, as neurons were never tested at contralateral ear intensities below +10 dB re threshold.

2.5 Data analysis

To obtain a time-intensity trading ratio, I compared the horizontal shift along the ITD axis of two ITD functions collected at different IID values, where stimulus intensity is held in one ear and increased in the other ear. Previous studies have measured the horizontal distance of the slopes fitted to the maximum and minimum points on each ITD function, closest to the biologically relevant range of ITDs for the animal tested (Fuzessery, 1997; Pollak, 1988). To account for the entire shape of an ITD function, I measured the horizontal distance between the inflection points of two ITD functions fitted to a four-parameter sigmoid function, similar to that of previous studies on binaural cues in the IC (Sayegh et al., 2014; Karcz et al., 2012; Tollin et al., 2008). The sigmoid function followed the equation:

$$f(x) = \left(\frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}} \right) + D$$

where *x* is the ITD value tested, *A* is the minimum asymptote, *B* is the slope factor, *C* is the horizontal midpoint, and *D* is the maximum asymptote of the sigmoid function. Because ITDs could be measured as negative values when the ipsilateral stimulus led the contralateral stimulus, we first shifted all ITD values by a constant to eliminate negative ITD values before fitting the data. After fitting, we subtracted the constant from the C value to obtain the function's midpoint. This method worked best for neurons with monotonic or near-monotonic ITD functions and the majority of neurons tested satisfied this condition. While holding the stimulus intensity at one ear constant, I varied the

intensity in the opposite ear. I then measured the horizontal shift between the ITD functions generated (i.e., the difference in the C value obtained from each ITD function) and divided this shift by the intensity difference between the two functions to obtain a time-intensity trading ratio, measured in μs/dB. I recorded the averaged time-intensity trading ratio for each neuron across a maximum intensity difference of ±21 dB in 3-6dB increments.

Unless stated otherwise, all data are reported as the mean ± standard error (SE). For statistical comparisons, the mean ± standard deviation (SD) is reported. A one-way analysis of variance test was used to compare a neuron's BEF across ITD function types. Pearson r correlations were used to test the relationship between a neuron's BEF and its dynamic response range, percent change in maximum response, best ITD, and time-intensity trading ratios. An independent t-test was used to compare BEF between ITD-selective and ITD-non-selective neurons, as well to compare time-intensity trading ratios measured when holding the stimulus intensity constant in the ipsilateral vs. contralateral ear. All statistical analyses were performed in SPSS or Python with an experiment-wise error rate of $\alpha = 0.05$.

3. Results

Single unit extracellular recordings were obtained from 80 IC neurons. The range of BEFs measured across all cells ranged from 22-66 kHz with a mean BEF of 39.5 ± 11.7 kHz. All 80 neurons were tested for ITD sensitivity and time-intensity trading, whereas 55 of the 80 were tested for IID sensitivity and binaural response characteristics

(E/E, E/I, E/O). Table 1 summarizes the proportion of neurons tested that were sensitive to either or both ITD and IID.

3.1 IID sensitivity

Consistent with past studies on IID sensitivity in the IC of bats, I identified two major types of IID selectivity (Sayegh et al., 2014). Of 55 neurons tested for IID, 22 (40%) displayed monotonic-type IID sensitivity, defined as a mostly linear decrease in spiking activity with increasing ipsilateral stimulation to below 50% of the cell's maximum response (Figure 2A). Two neurons (3.6%) displayed monotonic-type IID sensitivity, but with increased spiking with increasing ipsilateral stimulation (Figure 2B). Twelve neurons (21.8%) displayed peak-type IID sensitivity, defined as a response where the cell's activity remains below 50% of its maximum response, but peaks above 50% at a specific range of ipsilateral intensities (Figure 2C). Three neurons (5.5%) displayed some combination of these IID responses, depending on the intensity stimulated at the ipsilateral ear, and were classified as mixed-type (Figure 2D). The remaining 16 neurons (29.1%) displayed activity that remained consistently above 50% of the cell's maximum response and were determined not to be an IID-selective. Hence, a total of 39/55 (70.9%) neurons tested exhibited some form of IID sensitivity (Table 1).

By also testing the monaural response of the ipsilateral ear, I was able to identify the binaural response characteristics of these cells. I identified 17/55 (30.9%) neurons as EE, 28/55 (50.9%) neurons as EI, 7/55 (12.7%) neurons as EO, and 3/55 (5.5%) neurons with a mixed response. Table 2 summarizes the cells' binaural response characteristics

and their relation to the IID sensitivities tested. EE and EO neurons tended to be non-selective to IIDs, whereas EI neurons tended to display monotonic IID sensitivity with a minority showing peaked selectivity.

3,2 ITD sensitivity

3.2.1 Function types

Similar to previous studies (Fuzessery, 1997; Sayegh et al., 2014), I observed three different types of ITD-selective functions: peaked, monotonic, and U-shaped. Monotonic functions decreased with increasingly negative (i.e., ipsi-leading) ITDs, falling below 50% of the cell's maximum response (Figure 3A). Cells with peaked selectivity functions showed a distinct peak of activity that decreased below 50% of the cell's maximum response at both increasingly negative and increasingly positive ITDs (Figure 3B). U-shaped functions were similar to monotonic functions, but also increased their response to above 50% of the cell's maximum response at more negative ITDs (Figure 3C). Neurons that kept their response above 50% or cycled repeatedly above and below 50% of the cell's maximum response were classified as non-selective (Figure 3D). The most common ITD classification was monotonic (27/52; 51.9%), followed by U-shaped (15/52; 28.8%) and then peaked (10/52; 19.2%). From the 52 neurons that displayed ITD sensitivity, 34 (65.4%) were ITD sensitive at an IID of 0, whereas 18 (34.0%) only showed ITD sensitivity at typically negative IID values. This latter finding indicates that ipsilateral stimulation is necessary for ITD sensitivity in some IC neurons (e.g., Figure 4). There was no relationship between a cell's ITD selectivity function type

and its BEF ($F(2,45) = 2.87$, $p = .067$). However, there was a significant difference between the BEFs of ITD-selective ($\bar{x}$ = 37.4 kHz, SD = 9.7 kHz) and ITD-non-selective ($\bar{x}$ = 43.4 kHz, SD = 10.8 kHz) ITD neurons ($t(78) = -2.561$, $p = 0.012$). However, BEFs of selective ITD neurons were more heavily skewed (0.682) than non-selective ITD neurons (0.120) suggesting that this effect may be attributed to a smaller number of cells detected overall beyond 50 kHz (Figure 5). Fuzessery (1997) found a similar frequency dependence to ITD sensitivity in the IC of the pallid bad—although at lower BEFs overall—where most non-cyclical ITD sensitive neurons were tuned to frequencies below 28 kHz.

3.2.2 Dynamic response range

As reported in previous studies (Fuzessery, 1997; Kelly and Phillips, 1991), the dynamic response is the range of ITD values over which a cell's response decreased from 90% to 10% of its maximum response (Figure 6). The extent to which this response occurs over the biologically relevant range of ITDs for an animal is calculated as a percent change in maximum response and has been used as a measurement of a cell's ability to encode behaviorally relevant ITDs.

Of the 34 neurons that showed ITD sensitivity at an IID of 0 dB, nine were most sensitive outside the behaviorally relevant range of ±50 μs, and thus displayed 0% change in maximum response over the behaviorally relevant ITD range. For the remaining 25 cells, I determined their maximum and minimum response points to help define their dynamic response range. These were defined as the maximum and minimum responses of

the neuron that were closest to the behaviorally relevant ITD range. On average, the response maximum occurred at 236 ± 42.3 μs and the response minimum occurred at -224 ± 41.6 μs. Most responses appeared to be restricted to specific ITD values just outside the behaviorally relevant ITD range (Figure 7).

The average 10-90% response range was 368 ± 55.8 μs, (range = 120-1320 μs; n = 25). Over the behaviorally relevant range, the average percent change in maximum response was 35.8 ± 3.4% (range = 7.6-70.8%). On average, the dynamic response tended to be centered within the behaviorally relevant ITD range, with a mean midpoint of 6 ± 23.4 μs. Thus, we can expect the average span of the dynamic response of these neurons to be ± 184 μs, which is 3.7 times the behaviorally relevant range of ITDs in *E. fuscus*.

Previous studies have described a negative relationship between the best (maximum) ITD response and BEF, with the best ITD tending to occur closer to 0 μs at increasingly higher BEFs (McAlpine et al., 2001; McAlpine et al., 1996). However, we found no correlation between a cell's best ITD and its BEF in the IC of *E. fuscus* ($r = -0.351$, $n = 25$, $p = 0.086$; Figure 8).

A previous report on the pallid bat ID found that cells sensitive to phase cues showed a positive relation between the dynamic range and BEF, with cells tuned to higher BEFs having broader dynamic ITD ranges (Fuzessery, 1997). However, we found no correlation between BEF and dynamic response range ($r = -0.264$, $n = 25$, $p=.202$), nor between BEF and percent change ($r = 0.198$, $n = 25$, $p = 0.342$). That no relationship exists between the dynamic ITD range and BEF suggests that the onset time cue, rather

than the ongoing phase cue, may have been the dominant cue used by high-frequency-tuned neurons to calculate ITDs.

3.2.3 Time-intensity trading

To investigate the relationship between IID and ITD sensitivity, I calculated time-intensity trading ratios for 34 of the 52 neurons that demonstrated sensitivity to ITDs (Table 2). Larger trading ratios indicate a greater influence of IID on ITD processing in a cell, as small changes in dB would be expected to greatly shift the ITD function away from the behaviorally relevant range for the animal. Similarly, smaller trading ratios indicate a greater independence of ITD processing over IID processing in a cell, as variations in dB cause smaller shifts in the ITD function, allowing it to remain tuned to behaviorally relevant ranges.

An example calculation of time-intensity trading ratios is outlined in Figure 9. The mean time-intensity trading ratios measured across all neurons was 30.2 ± 3.5 μs/dB, ranging between 2.5 μs/dB and 83.2 μs/dB, with a bias toward trading ratios ≤39 μs/dB. The distribution of trading ratios is shown in Figure 10. There was no significant difference between trading ratios measured while keeping the contralateral ear intensity constant versus trading ratios measured while keeping the ipsilateral ear intensity constant ($t(53) = 0.684$, $p = 0.52$). Unlike studies on low-frequency tuned IC neurons in the cat, which predict a trend of increasing trading ratio with increasing BEF (Yin and Kuwada, 1983) there was no correlation between trading ratio and BEF ($r = -0.25$, $n = 34$, $p = 0.892$) from neurons in the IC of *E. fuscus*.

4. Discussion

4.1 Significance of studying transient onset ITD

Research on the neurophysiology of onset ITDs in sound localization has been scarce, with most studies instead focusing either on sensitivity to ongoing phase differences in the amplitude modulated envelope (Fuzessery, 1997) or stimulus carrier frequency (for review, see Grothe et al., 2010). This is likely because ongoing ITDs are a more salient and effective cue in azimuthal sound localization in humans than onset ITDs (Tobias & Shubert, 1959; Buell et al., 1991). The evidence is clear for low frequency stimuli, where ongoing interaural phase differences can be compared along the fine structure of the stimulus waveform, creating cycle-by-cycle temporal disparities for the length of the stimulus duration (Wightman and Kistler, 1992). This is also possible in amplitude modulated high-frequency stimuli, where cycle-by-cycle comparisons can be made in the phase difference of the stimulus envelope (Buell et al., 1991). But more recent psychoacoustics research in humans has found that the transient onset difference of a stimulus becomes more relevant in sound lateralization for short duration high frequency sounds. For example, Buell et al. (2008) demonstrated this by comparing the potency of transient onset disparities to ongoing phase differences in high frequency amplitude modulated stimuli. They determined that onset and ongoing interaural delays can be processed independently of each other, and that ongoing delays become less effective in sound lateralization at higher amplitude modulation rates and shorter stimulus durations, while the effectiveness of onset disparities increased. This implies that the salience of onset disparities is enhanced in stimuli with high amplitude modulation rates,

as the interaural delays between ongoing phase differences in the stimulus envelope become reduced with increasing modulation rate. It thus follows that onset disparities should also become more relevant when localizing high frequency pure tones, where the high oscillation rate in the stimulus waveform eliminates the reliability of ongoing phase differences, leaving onset cues to dominate ITD processing.

The present study demonstrates this salience to onset disparities by showing that neurons in the IC of *E. fuscus* are responsive to small changes in stimulus onset at high frequencies (Figures 3, 5, 6). Evidence that the salience of this transient cue would be overshadowed by the dominance of phase differences at low frequency stimuli has also been demonstrated in cats. Kuwada and Yin (1983) found that low-frequency-tuned neurons in the cat IC were sensitive to ongoing phase differences in low frequency stimuli but displayed no sensitivity to transient onset disparities when independently controlling for phase differences. This is possibly because the ongoing phase difference was still available in the low frequency waveform of the stimulus. Thus, the ongoing cycle-by-cycle phase difference of zero in this stimulus dominated over any transient time disparities at the onset of the stimulus. A neuron's cyclical response to phase differences appears to be related to its BEF tuning, as even transient noise and click stimuli are capable of eliciting cyclical ITD functions in neurons tuned to low-frequency stimuli (Carney and Yin, 1989). Although the majority of our analysis on ITD sensitivity was at the microsecond scale, within the range of ± 1000 μs, I did not find evidence of cyclical activity when testing for ITD sensitivity at longer ITD ranges (Figure 11). Previous research has shown that even when neurons show cyclical ITD functions, the frequency

of those cycles is unrelated to the frequency of stimulation (Sayegh *et al.*, 2014). I also did not observe any relationship between a neuron's BEF with any of our measurements of ITD characteristics (dynamic range, best ITD, time-intensity trading ratios) or classifications (monotonic, peaked, U-shape). By isolating neurons tuned to high BEFs and studying ITDs using high frequency pure tones, I am confident that the neural responses I observed reliably reflect sensitivities to onset time differences.

4.2 Comparison to other species

The only previous study on ITD sensitivity in *E. fuscus* also demonstrated that the IC of this species is sensitive to ITDs but did not delve into a detailed analysis of its ITD tuning characteristics or its relation to IID sensitivity (Sayegh et al., 2014). A neurophysiological study on the MSO in *E. fuscus* revealed that 54% of neurons received only monaural contralateral excitatory input (EO), while 22% of neurons received binaural excitatory inputs (EE) and 17% of neurons received contralateral excitatory and ipsilateral inhibitory inputs (EI), although the MSO's ITD sensitivity was not tested (Grothe et al., 2001). This contrasts to cells in the IC of this bat according to findings in Sayegh et al. (2014) as well our own study. I found that only 13% of neurons were EO (monaural), 31% were EE, and 51% were EI. This high proportion of binaurally sensitive neurons in the IC of *E. fuscus* are closer to the proportion of binaurally sensitive, neurons found in the MSO of the free-tailed bat, although the IC in *E. fuscus* appears to have more EI sensitive neurons and fewer EE sensitive neurons than the MSO of the free-tailed bat (Grothe and Park, 1998). Nevertheless, onset ITD sensitivity has been observed in the IC of the free-tailed bat as well, although using short duration frequency-modulated sweep

stimuli instead of pure tones (Pollak 1988). Altogether, this suggests that ITD sensitivity in the IC of bats may not necessarily depend on MSO neurophysiology. However, a detailed analysis of ITD sensitivity in the IC of the free-tailed bat has not been conducted, leaving the possibility that MSO neurophysiology may affect the tuning of ITD sensitivity in the IC (e.g., dynamic response range). The only other available comparison between these bat species is that the time-intensity trading ratio of 30 μs/dB in *E. fuscus* is smaller than the 47 μs/dB trading ratio in the IC of the free-tailed bat (Pollak 1988). There appears to be a greater proportion of neurons (33%) with short time-intensity trading ratios (≤19 μs/dB) in the IC of *E. fuscus* than in the free-tailed bat IC (≤5%). Having a smaller trading ratio suggests that IID cues may have less influence on ITD sensitivity in *E. fuscus* compared to the free-tailed bat. The trading ratio found in *E. fuscus* is also similar to that of the molossus bat (Harnischfeger et al., 1985) although that study found far fewer neurons sensitive to onset ITDs in the IC and included neurons from SOC nuclei in its analyses, making it difficult to compare between the two species.

ITD sensitivity appears to be very similar between *E. fuscus* and the pallid bat (Fuzessery 1997). The pallid bat is reported to have an average dynamic response range of 304 μs (range: 125-625 μs) with 37% change in maximum response over its behaviorally relevant range. This is comparable to the dynamic response range of 368 μs (range: 120-1320 μs) with 36% change over the behaviorally relevant range found in *E. fuscus*. In contrast, the pallid bat IC had a smaller average time-intensity trading ratio of 18 μs/dB, ranging between 7.5 to 30 μs/dB. In *E. fuscus*, time-intensity trading ratios ranged between 2.5 to 83 μs/dB, although with a majority (76%) of neurons tuned to

trading ratios below 40 µs/dB (Figure 10). Smaller trading ratios in the pallid bat may be related to its gleaning foraging behavior, where it relies on passive sound localization while gleaning ground-dwelling prey, compared to *E. fuscus* that uses high-frequency echolocation to hunt flying prey. Aerial insectivorous bats likely evolved a greater need to localize high frequency sounds, where IIDs dominate, compared to passive gleaners that listen for low frequency prey sounds, where ongoing ITDs would dominate (Bell, 1982; Orr, 1954).

Both the pallid bat and *E. fuscus* demonstrate a larger percent change in maximum response over their respective behaviorally relevant ITD range when compared to the 29% change observed in neurons of the auditory cortex of the rat (Kelly and Phillips, 1991). Both bats also demonstrate a shorter dynamic response range compared to neurons in the rat IC, which was 570 µs (Kidd and Kelly, 1996). These findings strengthen the interpretation by Fuzessery et al. (1997) that, as a result of having a smaller head size, certain bats' nervous systems might compensate for the reduction in behaviorally relevant ITDs available to them by sharpening their dynamic response to ITDs. A decrease in the dynamic response range helps achieve this by allowing more of the dynamic portion of the ITD function to fall within the behaviorally relevant range of ITDs. Indeed, the range of percent changes observed in the IC of *E. fuscus* (8-71%) are comparable to those observed in the gerbil MSO (30-85%), a commonly studied species for mammalian ITD sensitivity (Brand et al., 2002). This range increases slightly to 11-75% when using a more liberal behaviorally relevant range of ±75 µs, as calculated by Ayetkin et al., (2004).

Psychoacoustical studies on bats' testing their sensitivity to transient onset ITDs are needed to confirm behavioral sensitivity.

4.3 Behavioral significance

Despite clear sensitivity to transient onset ITDs observed in the IC, it is difficult to assert that *E. fuscus* would rely significantly on ITDs for sound localization based on neurophysiological data alone. It is important to consider that although 52 (65%) IC neurons tested displayed some form of ITD sensitivity to at some IID level, this number dropped to 34 (43%) when controlling for stimulus intensity at either ear by testing the neuron at an IID of 0 dB. This number falls further to 25 (31%) when only counting neurons whose dynamic range passed through the behaviorally relevant range of ITDs.

In contrast, over 70% of IC neurons in *E. fuscus* displayed sensitivity to IIDs, almost all of which spanned 100% of the bat's behaviorally relevant range of IIDs (± 20 dB). Thus, in *E. fuscus,* the proportion of neurons sensitive to IIDs in the IC is much larger than the proportion sensitive to ITDs. It is also important to consider that naturally localizing free-field sounds will cause both ITDs and IIDs to vary simultaneously. The degree to which ITDs are influenced by IIDs is reflected in the average time-intensity trading ratio of the animal, which was 30 μs/dB in *E. fuscus*. This number indicates that every dB change in stimulus intensity difference at either ear, the cell's ITD tuning will shift by ~30 μs. Therefore, even small IID changes of 3-6dB are enough to cause the ITD tuning of IC neurons to shift outside the behaviorally relevant range of the animal (Figures 4 and 9). This finding, coupled with the relative proportion of ITD and IID

sensitive neurons in the IC, strongly suggests that the IC of *E. fuscus* is more reliably tuned to IIDs than ITDs. This interpretation follows that of previous research on ITD sensitivity in bats' IC neurons (Pollak, 1988; Fuzessery, 1997; Sayegh et al., 2014).

The current evidence does not suggest that onset ITDs serve as a dominant cue in azimuthal sound localization in bats, but rather that they serve as a weak binaural cue that could contribute to overall azimuthal localization performance, as has been demonstrated in human psychoacoustical studies (Buell et al; 1991; Buell et al., 2008). The utility of onset ITDs becomes apparent when examining bats' behavioral performance to sound lateralization at low frequencies, where IIDs become less relevant. Koay et al. (1998) demonstrated that *E. fuscus* was unable to localize low frequency stimuli in a conditioned avoidance task, suggesting that they do not use the ongoing phase cue in stimulus lateralization. However, these animals continued to perform above chance when tested with stimuli between 11-22 kHz. For *E. fuscus*, with an average interaural distance of 12.08 ± 0.84 mm (Sayegh et al., 2014), the IID cue is expected to become much weaker at frequencies below approximately 28 kHz, where the stimulus wavelength exceeds the animal's interaural distance, thereby reducing the effect of a sound shadow in creating intensity disparities. In the absence of both IIDs and ongoing ITDs, it is possible that *E. fuscus* was able to perform above chance using the transient onset ITD cue. If the animal was indeed insensitive to ongoing phase differences, then this further suggests that it should be able to discriminate the transient onset disparities of a stimulus, as the ongoing ITD cues should not be able to dominate over the transient onset ITD cue. In Norway rats, shortening the duration of a low frequency (1 kHz) tone improved rather than hindered

the animals' ability to localize a sound stimulus, suggesting sensitivity to the transient onset cue rather than the ongoing phase cue (Wesolek et al., 2010). Future behavioral work should test the potency of onset cues in bats by independently varying onset and ongoing ITDs in psychoacoustical testing.

4.3 Mechanisms of ITD tuning

The traditional "place theory" model for how the nervous system interprets ITDs in the brain was proposed by Jeffress (1948), who suggested that an array of cells with delay lines act as a coincidence detector of binaural inputs arriving from each ear. The model proposed that inputs from either ear would travel along delay lines of varying lengths before converging onto an array of cells, where the arrival of coincident inputs would maximally excite a particular cell in the array. Each cell would thus be maximally sensitive to a particular timing difference between the acoustic delays from each ear. Single cell recordings should thus reveal peak activity for any given cell at various different ITDs–presumably within the behaviorally relevant range of ITDs for the animal–with each peak representing the cell's tuning to a particular azimuthal angle. While the neuronal circuitry required to support this model has been confirmed in the avian nervous system (Köppl and Carr 2008; Carr and Koshini, 1988; Seidl et al., 2010), evidence in the mammalian nervous system has either been lacking or inconsistent across species (for review, see McAlpine and Grothe, 2003; Ashida and Carr, 2011; Grothe and Pecka; 2014).

Neurophysiological studies in the MSO of mammals has revealed that the peaks of ITD tuning functions tend to occur just outside the animal's behaviorally relevant range of ITDs (Grothe et al., 2010). As a result, the steepest portion of the ITD function tends to be centered within the behaviorally relevant range. This has led to an alternative theory for ITD coding in mammals, which suggests that neurons code for the difference in activity along the slope of an ITD function, rather than at the place of its peak activity. Anatomical and physiological evidence in the MSO of gerbils (Lesica et al., 2010; Brand et al., 2002) and the IC of guinea pigs (Palmer et al., 1990) and cats (Yin et al., 1986) and gerbils (Volmer, 2018) seem to support this slope-coding model. The present results demonstrate the first evidence in support of the slope coding model for transient onset ITDs in the IC of bats. The peak ITD response in the IC of *E. fuscus* tends to occur within a narrow range of ITDs, slightly contralateral to the behaviorally relevant range and always outside of it (Figure 7).

Interestingly, I found no correlation between a neuron's BEF and its best ITD (Figure 8). This is in contrast to previous studies in lower frequency hearing mammals, which found that the best ITD of a neuron tends to approach 0 with increasingly higher BEF (McAlpine et al., 2001; McAlpine et al., 1996; Vollmer 2018). The lack of shift may be due to differences in stimulus used across species, as previous research used broadband noise or low frequency pure tones, whereas our study used high frequency pure tones, between 20-60kHz. Furthermore, our results reflect binaural tuning to the transient onset portion of a tone, and not the ongoing phase differences of the stimulus. It should thus be an expectation that the ITD data I collect would be invariant to stimulus

frequency. Indeed, Vollmer (2018) reported that, across a range of stimulus frequencies, the peak ITD response for a cell tended to be most similar (i.e., less variable; sharply tuned to similar peak ITD values) in transient click/chirp stimuli, and least similar (i.e., more variable; broadly tuned to peak ITD values) for tone stimuli, further suggesting an invariance to stimulus frequency in ITD coding for transient stimuli.

However, it is also possible that any frequency dependence becomes less potent at high frequencies. This is further supported by evidence from guinea pigs, where an exponential decay function relates BEF to best ITD in IC neurons (McAlpine et al., 1996). McAlpine et al. (2001) also showed that low frequency stimuli peaked over a wider range of ITDs than high frequency stimuli. This suggests that any trend relating BEF to best ITD in high frequency stimuli–on the order of tens of kHz–may be too small to be significant.

Despite its invariance to stimulus frequency, the 236 µs average best ITD in *E. fuscus* is comparable to findings in the cat IC (Yin et al., 1986), guinea pig IC (McAlpine at al., 2001; Palmer et al., 1990), and gerbil MSO (Brand et al., 2002), suggesting that neural tuning of the auditory system to best ITDs might be preserved across mammalian species.

Additionally, the observation that some neurons only showed ITD tuning at certain negative IIDs, when the ipsilateral intensity was higher than the contralateral intensity (Figure 4), supports the assertion that inhibition is important in tuning a cell's sensitivity to ITDs (Pecka et al., 2008; Grothe and Pecka; 2014). Indeed, our time-

intensity trading results reveal that increasing the stimulus intensity at the ipsilateral ear tends to shift the ITD function toward contralateral-leading ITDs, supporting the notion that fine tuning of inhibition could work to shift a cell's best ITD toward contralateral delays.

4.4 Summary and conclusions:

Our results demonstrate the existence of high frequency neurons in the IC of the echolocating bat, *Eptesicus fuscu*s, that display some form of sensitivity to transient onset ITDs. ITD tuning characteristics were invariant to the cell's BEF, indicating that the cells are likely responding to transient onset time differences rather than ongoing phase differences within the waveform of the high frequency tone. This indicates that the IC in this bat species possess the capacity to process onset time disparities within behaviorally relevant ranges, which may assist in the bat's ability to localize sound in space, independent of phase differences in the carrier frequency. Our results are comparable to ITD sensitivities found in various midbrain structures and brainstem nuclei of other mammals. Future studies should explore how high frequency selective cells in the IC respond independently to onset and ongoing ITDs. This should be coupled with behavioral studies that independently vary onset and phase differences to elucidate any possibility that these cells are responding to phase differences.

References

Ashida, G., & Carr, C. E. (2011). Sound localization: Jeffress and beyond. *Current Opinion in Neurobiology*, *21*(5), 745–751.

Aytekin, M., Grassi, E., Sahota, M., & Moss, C. F. (2004). The bat head-related transfer function reveals binaural cues for sound localization in azimuth and elevation. *The Journal of the Acoustical Society of America*, *116*(6), 3594–3605.

Bell, G. P. (1982). Behavioral and ecological aspects of gleaning by a desert insectivorous bat Antrozous pallidus (Chiroptera: Vespertilionidae). *Behavioral Ecology and Sociobiology*, *10*(3), 217–223.

Brand, A., Behrend, O., Marquardt, T., McAlpine, D., & Grothe, B. (2002). Precise inhibition is essential for microsecond interaural time difference coding. *Nature*, *417*, 543–547.

Buell, T. N., Griffin, S. J., & Bernstein, L. R. (2008). Listeners' sensitivity to "onset/offset" and "ongoing" interaural delays in high-frequency, sinusoidally amplitude-modulated tones. *The Journal of the Acoustical Society of America*, *123*(1), 279–294.

Buell, T. N., Trahiotis, C., & Bernstein, L. R. (1991). Lateralization of low-frequency tones: Relative potency of gating and ongoing interaural delays. *The Journal of the Acoustical Society of America*, *90*(6), 3077–3085.

Carney, L. H., & Yin, T. C. (1989). Responses of low-frequency cells in the inferior colliculus to interaural time differences of clicks: excitatory and inhibitory components. *Journal of Neurophysiology*, *62*(1), 144–161.

Carr, C. E., & Konishi, M. (1988). Axonal delay lines for time measurement in the owl's brainstem. *Proceedings of the National Academy of Sciences*, *85*(21), 8311–8315.

Covey, E. (2005). Neurobiological specializations in echolocating bats. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*, *287*(1), 1103–1116.

Covey, E., & Casseday, J. H. (1995). The lower brainstem auditory pathways. In *Hearing by bats* (pp. 235-295). Springer, New York, NY.

Dietz, M., Marquardt, T., Salminen, N. H., & McAlpine, D. (2013). Emphasis of spatial cues in the temporal fine structure during the rising segments of amplitude-modulated sounds. *Proceedings of the National Academy of Sciences*, *110*(37), 15151–15156.

Ehrlich, D., Casseday, J. H., & Covey, E. (1997). Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *Journal of Neurophysiology, 77*(5), 2360–2372.

Fuzessery, Z. M. (1997). Acute sensitivity to interaural time differences in the inferior colliculus of a bat that relies on passive sound localization. *Hearing Research*, *109*(1-2), 46–62.

Fuzessery, Z. M., & Pollak, G. D. (1985). Determinants of sound location selectivity in bat inferior colliculus: a combined dichotic and free-field stimulation study. *Journal of Neurophysiology*, *54*(4), 757-781.

Grothe, B., & Park, T. J. (1995). Time can be traded for intensity in the lower auditory system. *Naturwissenschaften*, *82*(11), 521–523.

Grothe, B., & Park, T. J. (1998). Sensitivity to interaural time differences in the medial superior olive of a small mammal, the Mexican free-tailed bat. *The Journal of Neuroscience*, *18*(16), 6608–6622.

Grothe, B., & Park, T. J. (2000). Structure and function of the bat superior olivary complex. *Microscopy Research and Technique*, *51*(4), 382–402.

Grothe, B., & Pecka, M. (2014). The natural history of sound localization in mammals–a story of neuronal inhibition. *Frontiers in Neural Circuits*, *8*, 116.

Grothe, B., Pecka, M., & McAlpine, D. (2010). Mechanisms of sound localization in mammals. *Physiological Reviews*, *90*(3), 983–1012.

Grothe, B., Covey, E., & Casseday, J. H. (2001). Medial superior olive of the big brown bat: neuronal responses to pure tones, amplitude modulations, and pulse trains. *Journal of Neurophysiology*, *86*(5), 2219–2230.

Harnischfeger, G., Neuweiler, G., & Schlegel, P. (1985). Interaural time and intensity coding in superior olivary complex and inferior colliculus of the echolocating bat *Molossus ater*. *Journal of Neurophysiology*, *53*(1), 89–109.

Heffner, H. E., & Heffner, R. S. (2016). The evolution of mammalian sound localization. *Acoustics Today*, *12*(1), 20–35.

Heffner, R. S., Koay, G., & Heffner, H. E. (1999). Sound localization in an Old-World fruit bat (*Rousettus aegyptiacus*): acuity, use of binaural cues, and relationship to vision. *Journal of Comparative Psychology*, *113*(3), 297.

Heffner, R. S., Koay, G., & Heffner, H. E. (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*(1), 412–421.

Heffner, R. S., Koay, G., & Heffner, H. E. (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*(1), 42–52.

Jeffress, L. A. (1948). A place theory of sound localization. *Journal of Comparative and Physiological Psychology*, *41*(1), 35.

Joris, P. X., & Yin, T. C. (1995). Envelope coding in the lateral superior olive. I. Sensitivity to interaural time differences. *Journal of Neurophysiology*, *73*(3), 1043–1062.

Karcz, A., Rübsamen, R., & Kopp-Scheinpflug, C. (2012). Low-threshold potassium currents stabilize IID-sensitivity in the inferior colliculus. *Frontiers in Neural Circuits*, *6*, 60.

Kelly, J. B., & Phillips, D. P. (1991). Coding of interaural time differences of transients in auditory cortex of *Rattus norvegicus*: implications for the evolution of mammalian sound localization. *Hearing Research*, *55*(1), 39–44.

Kidd, S. A., & Kelly, J. B. (1996). Contribution of the dorsal nucleus of the lateral lemniscus to binaural responses in the inferior colliculus of the rat: interaural time delays. *The Journal of Neuroscience*, *16*(22), 7390–7397.

Koay, G., Kearns, D., Heffner, H. E., & Heffner, R. S. (1998). Passive sound-localization ability of the big brown bat (*Eptesicus fuscus*). *Hearing Research*, *119*(1-2), 37–48.

Köppl, C., & Carr, C. E. (2008). Maps of interaural time difference in the chicken's brainstem nucleus laminaris. *Biological Cybernetics*, *98*(6), 541–559.

Kuwada, S., & Yin, T. C. (1983). Binaural interaction in low-frequency neurons in inferior colliculus of the cat. I. Effects of long interaural delays, intensity, and repetition rate on interaural delay function. *Journal of Neurophysiology*, *50*(4), 981–999.

Lesica, N. A., Lingner, A., & Grothe, B. (2010). Population coding of interaural time differences in gerbils and barn owls. *The Journal of Neuroscience*, *30*(35), 11696–11702.

Li, N., Gittelman, J. X., & Pollak, G. D. (2010). Intracellular recordings reveal novel features of neurons that code interaural intensity disparities in the inferior colliculus. *The Journal of Neuroscience*, *30*(43), 14573–14584.

McAlpine, D., & Grothe, B. (2003). Sound localization and delay lines–do mammals fit the model? *Trends in Neurosciences*, *26*(7), 347–350.

McAlpine, D., Jiang, D., & Palmer, A. R. (1996). Interaural delay sensitivity and the classification of low best-frequency binaural responses in the inferior colliculus of the guinea pig. *Hearing Research*, *97*(1-2), 136–152.

McAlpine, D., Jiang, D., & Palmer, A. R. (2001). A neural code for low-frequency sound localization in mammals. *Nature Neuroscience*, *4*(4), 396.

Orr, R. T. (1954). Natural History of the Pallid Bat, *Antrozous pallidus*: (Le Conte). *Proceedings of the California Academy of Sciences*, *28*, 165–815.

Palmer, A. R., Rees, A., & Caird, D. (1990). Interaural delay sensitivity to tones and broad band signals in the guinea-pig inferior colliculus. *Hearing Research*, *50*(1-2), 71–86.

Pecka, M., Brand, A., Behrend, O., & Grothe, B. (2008). Interaural time difference processing in the mammalian medial superior olive: the role of glycinergic inhibition. *The Journal of Neuroscience*, *28*(27), 6914–6925.

Perrott, D. R. (1969). Role of signal onset in sound localization. *The Journal of the Acoustical Society of America*, *45*(2), 436–445.

Pollak, G. D. (1988). Time is traded for intensity in the bat's auditory system. *Hearing Research*, *36*(2–3), 107–124.

Pollak, G. D. (2012). Circuits for processing dynamic interaural intensity disparities in the inferior colliculus. *Hearing Research*, *288*(1-2), 47–57.

Rayleigh, L. (1907). XII. On our perception of sound direction. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science*, *13*(74), 214–232.

Sayegh, R., Aubie, B., & Faure, P. A. (2014). Dichotic sound localization properties of duration-tuned neurons in the inferior colliculus of the big brown bat. *Frontiers in Physiology*, *5*, 215.

Schofield, B. R., (2005) Superior olivary complex and lateral lemniscal connections of the auditory midbrain. In C. Schreiner & J. A. Winer (Eds.), *The Inferior Colliculus* (pp. 132-154). New York, NY: Springer.

Seidl, A. H., Rubel, E. W., & Harris, D. M. (2010). Mechanisms for adjusting interaural time differences to achieve binaural coincidence detection. *The Journal of Neuroscience*, *30*(1), 70–80.

Tobias, J. V., & Schubert, E. D. (1959). Effective onset duration of auditory stimuli. *The Journal of the Acoustical Society of America*, *31*(12), 1595–1605.

Tollin, D. J., Koka, K., & Tsai, J. J. (2008). Interaural level difference discrimination thresholds for single neurons in the lateral superior olive. *The Journal of Neuroscience*, *28*(19), 4848–4860.

Tollin, D. J., & Yin, T. C. (2005). Interaural phase and level difference sensitivity in low-frequency neurons in the lateral superior olive. *The Journal of Neuroscience*, *25*(46), 10648–10657.

Vollmer, M. (2018). Neural processing of acoustic and electric interaural time differences in normal-hearing gerbils. *Journal of Neuroscience*, 3328–17.

Wesolek, C. M., Koay, G., Heffner, R. S., & Heffner, H. E. (2010). Laboratory rats (*Rattus norvegicus*) do not use binaural phase differences to localize sound. *Hearing Research*, *265*(1-2), 54–62.

Wightman, F. L., & Kistler, D. J. (1992). The dominant role of low-frequency interaural time differences in sound localization. *The Journal of the Acoustical Society of America*, *91*(3), 1648–1661.

Yin, T. C., Chan, J. C., & Irvine, D. R. (1986). Effects of interaural time delays of noise stimuli on low-frequency cells in the cat's inferior colliculus. I. Responses to wideband noise. *Journal of Neurophysiology*, *55*(2), 280–300.

Zook, J. M., & Casseday, J. H. (1980). Identification of auditory centers in lower brain stem of two species of echolocating bats: evidence from injection of horseradish peroxidase into inferior colliculus. In *Proceedings of the Fifth International Bat Research Conference, edited by Wilson DE and Gardner AL (Texas Tech Press, Lubbock, TX:)* (pp. 51-59).

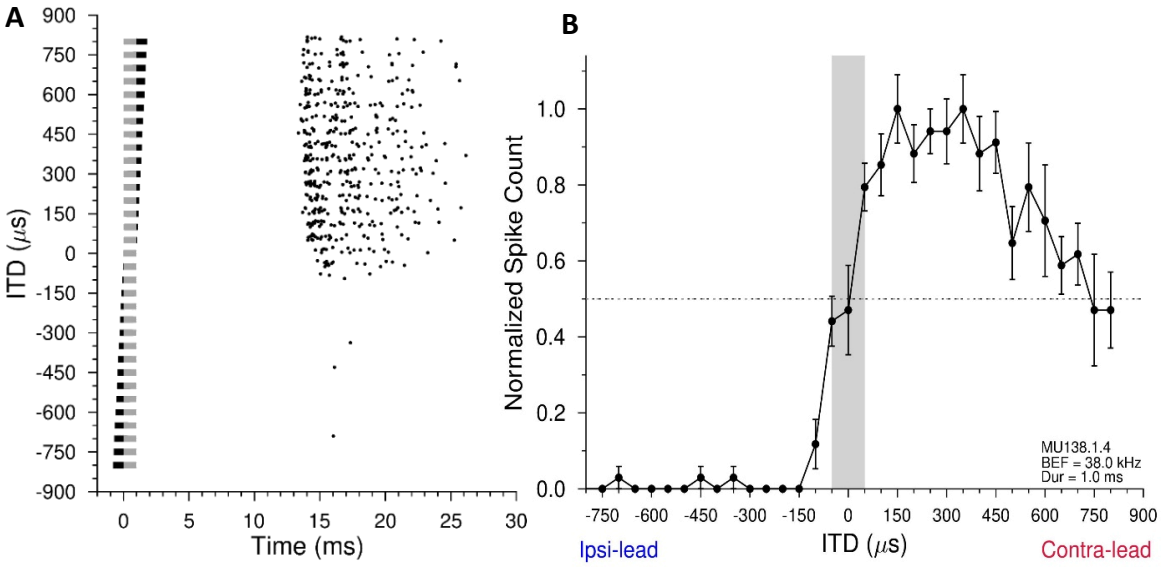

Fig 1: Visualization of stimulus paradigm used to measure neural ITD sensitivity. **(A)** Dot raster display of spikes evoked by the onset of contralateral stimulus at varying ITDs (μs). Bars represent the duration (1 ms) and onset of stimuli (black: ipsilateral stimulus; grey: contralateral stimulus). The ipsilateral stimulus was randomly varied in onset time (resolution = 50 μs) relative to a stationary contralateral stimulus, generating ITDs measured relative to the contralateral stimulus (i.e., contralateral onset time – ipsilateral onset time). Dots represent individual action potentials. **(B)** Normalized mean spike counts from panel A relative to maximum. Values represent average spike counts measured over 10-20 trials. Dotted line represents 50% of maximum response. Grey box represents the behaviorally relevant range of ITDs for *E. fuscus* (±50 μs). Error bars show standard errors of the mean. (BEF = Best excitatory frequency; Dur = Stimulus duration; Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference; MU138.1.4 = Animal ID).

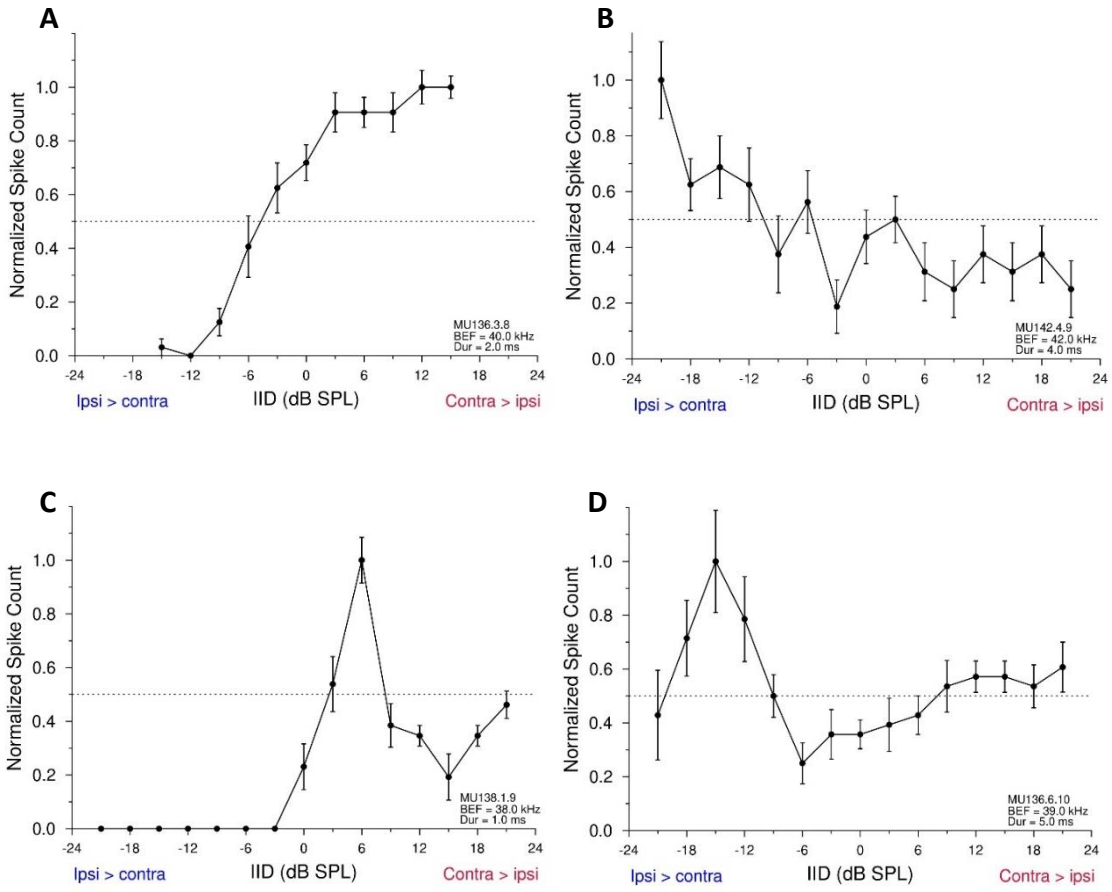

Fig 2: Example IID functions from for IC neurons in *E. fuscus.* All plots are mean ± SE spike count to various IIDs, normalized to the maximum response evoked by the neuron and measured across 10-20 trials. In all trials, a contralateral stimulus was kept stable at +10 dB re threshold of each individual cell while ipsilateral stimulus intensity was randomly varied in 3 dB steps within the range of ±21 dB re contra. IID value represents the intensity difference between the two stimuli, calculated as the contralateral stimulus intensity minus the ipsilateral stimulus intensity. Positive IID values indicates that the stimulus presented at the contralateral ear was more intense (larger dB value) than the stimulus presented at the ipsilateral ear. Negative IID values indicate the opposite

relationship. The dotted line represents 50% of the neuron's maximum response. **(A)** A monotonic IID-selective neuron that steadily decreased its activity in response to increasing ipsilateral stimulus intensities. **(B)** A reverse-monotonic IID-selective neuron that steadily increased its activity in response to increasing ipsilateral stimulus intensity. **(C)** A peaked IID-selective neuron that displayed a distinct peak of activity at an IID of +6 dB and steadily decreased its activity from this point in response to both increasing and decreasing ipsilateral stimulus intensities. **(D)** A mixed-type IID-selective neuron that demonstrated peaked-type IID selectivity at negative IIDs, but monotonic IID selectivity at more positive IIDs. its activity in response to increasing ipsilateral stimulus intensity (negative IIDs). (BEF = Best excitatory frequency; Dur = Stimulus duration; Ipsi = Ipsilateral; Contra = Contralateral; IID = Interaural intensity difference).

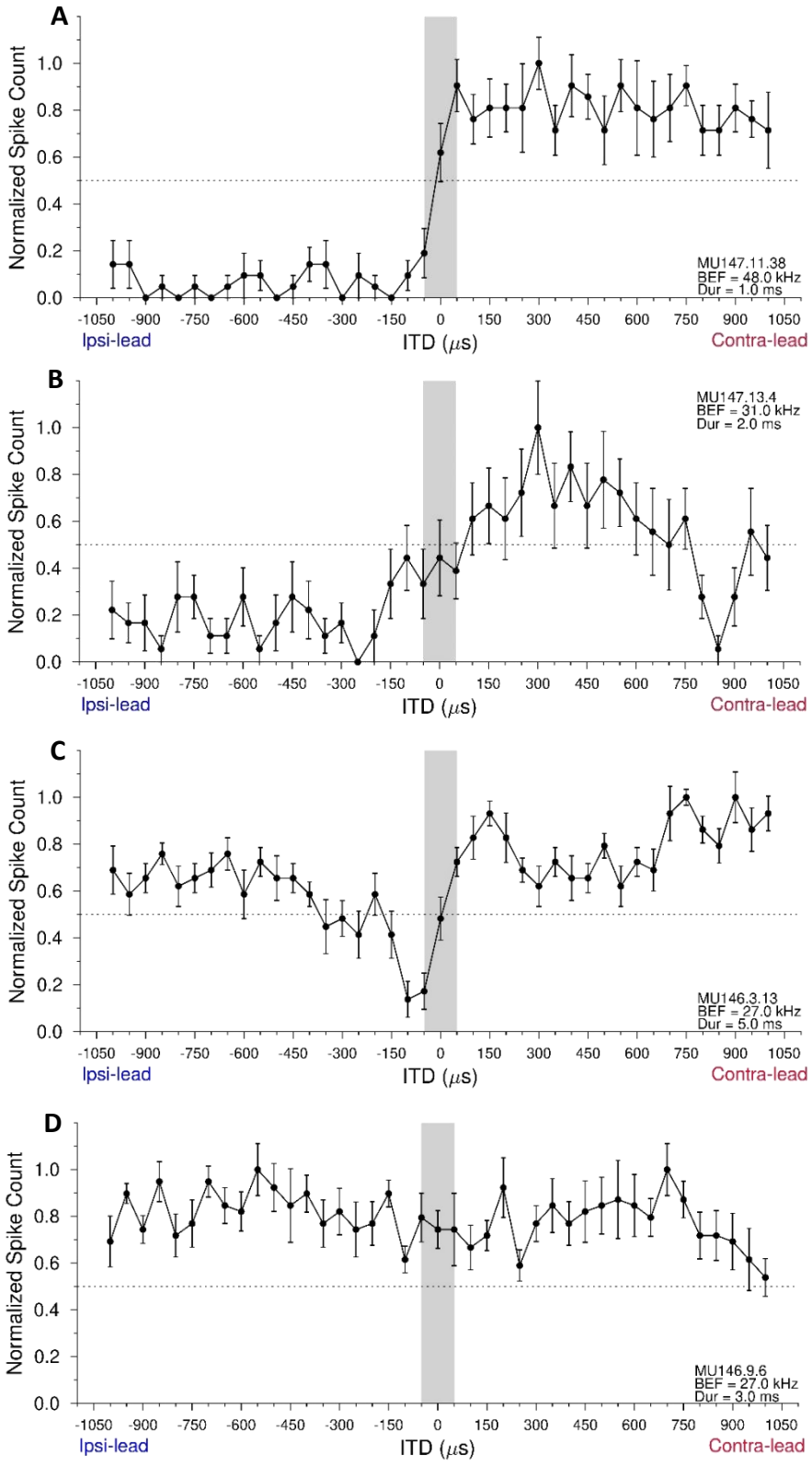
A
Normalized Spike Count
MU147.11.38
BEF = 48.0 kHz
Dur = 1.0 ms
Ipsi-lead
ITD (μs)
Contra-lead
B
MU147.13.4
BEF = 31.0 kHz
Dur = 2.0 ms
C
MU146.3.13
BEF = 27.0 kHz
Dur = 5.0 ms
D
MU146.9.6
BEF = 27.0 kHz
Dur = 3.0 ms

Fig 3: Example ITD functions measured from four neurons in the IC of *E. fuscus.* Plots represent show mean ± SE normalized spike count in response to various ITDs across 10-20 trials. Contralateral and ipsilateral stimulus intensity was kept stable at +10 dB (re contralateral threshold) for all trials while ITD was randomly varied in 50 µs steps within a range of ± 1000µs (see Fig. 1A for dot raster visualization). ITDs calculated as the contralateral stimulus onset time minus the ipsilateral stimulus onset time. The dotted line represents the 50% point of the neuron's maximum response. Grey box represents the behaviorally relevant range of ITDs for the bat (±50 µs). **(A)** A monotonic ITD-selective neuron that steadily decreased its activity in response to ipsilaterally-leading onsets times (i.e., negative ITDs). **(B)** A peaked ITD-selective neuron with a distinct increase in activity at an ITD of +300 µs that decreased its activity from this point in response to both ipsilaterally and contralaterally leading stimulus onsets. **(C)** A U-shaped ITD selective neuron that displayed a distinct trough of decreased activity for ITDs between -150 and 0 µs and increased its activity from this point in response to both ipsilaterally and contralaterally leading stimulus onsets. **(D)** A neuron that was non-selective to ITDs. This cell's response remained above 50% of its maximum response for ITDs. (BEF = Best excitatory frequency; Dur = Stimulus duration; Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference).

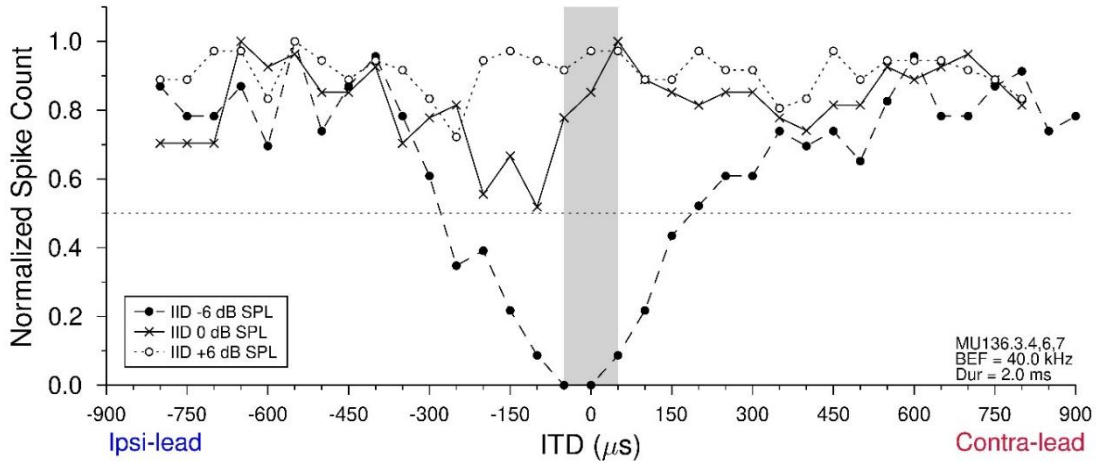

Fig 4: Example of a neuron that only displayed ITD sensitivity at certain IID values. Positive IIDs indicate an increase in the contralateral ear intensity while the ipsilateral ear intensity was held constant, whereas negative IIDs indicate an increase in the ipsilateral ear intensity while the contralateral ear intensity was held constant. The dotted line represents 50% of the neuron's maximum response. Grey bar represents the behaviorally relevant range of ITDs for the bat (±50 μs). At an IID of 0 dB (solid line, cross marker) and at an IID of +6 dB (dotted line, open circle marker) the cell's response remained above 50% of the maximum response and was not selective to changing ITDs. It is only at an IID of -6 dB (dashed line, filled circle marker) that the cell demonstrates U-shaped sensitivity to ITDs. (BEF = Best excitatory frequency; Dur = Stimulus duration; Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference).

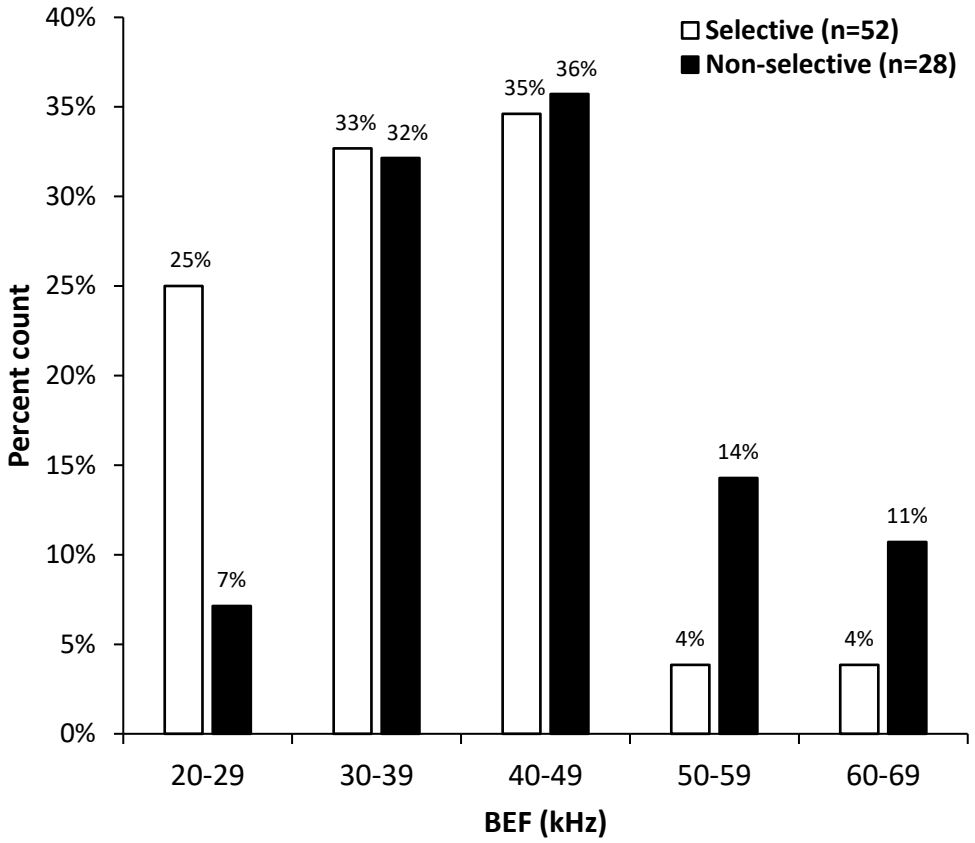

Fig 5: Histogram showing ITD selectivity as a function of BEF. White bars represent ITD selective cells (n = 52). Black bars represent cells that were non-selective to ITD (n = 28).

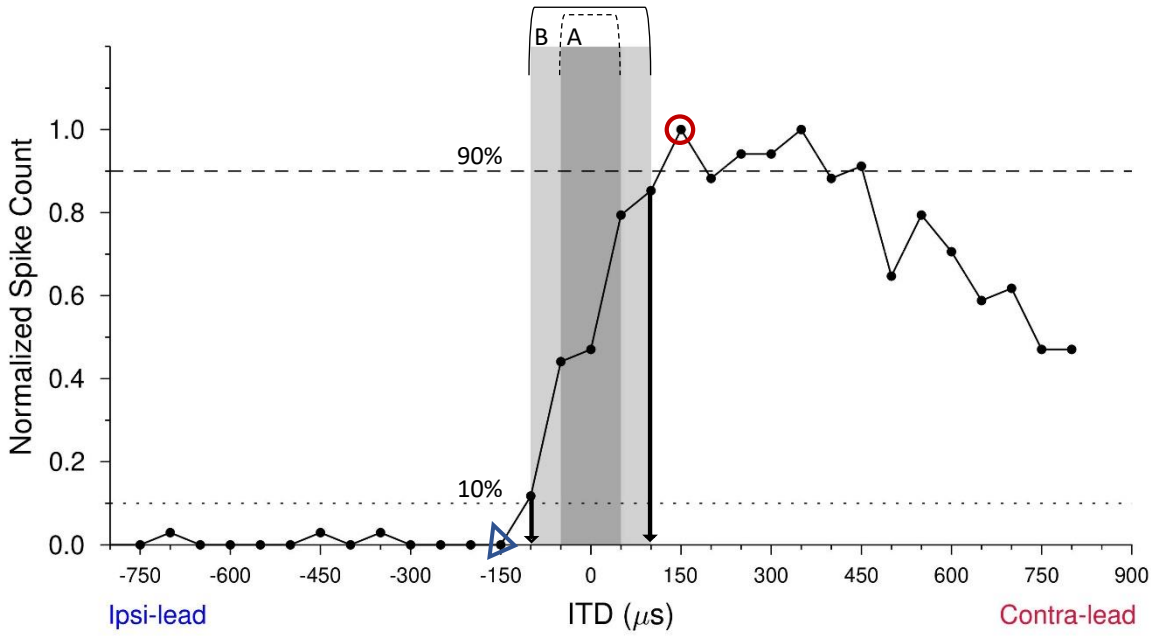

Fig 6: Measuring the dynamic response properties of ITD functions. Error bars removed for clarity. The response minima (open triangle) and response maxima (open circle) were determined as the minimum and maximum responses on the ITD function closest to the behaviorally relevant range (dark grey band [A]; ± 50 µs). The dynamic response range was the ITD value measured from 90% (dashed line) to 10% (dotted line) of the cell's spiking response, rounded to the nearest 50 µs, closest to the cell's behaviorally relevant range. The percent change in maximum response was calculated as the dynamic response range (light grey band [B]) over the behaviorally relevant range. In the example shown, the response maxima was +150 µs (open circle) and the response minima was -150 µs. The 90% response occurred at 100 µs and the 10% response occurred at -100 µs. Therefore, the dynamic range spanned 200 µs between the 90% and 10% points on the ITD axis. The percent change was calculated as the range of ITDs in the behaviorally relevant range (100 µs) divided by the dynamic range (200 µs) (i.e., A/B * 100%). This

would be 50%, indicating that 50% of the cell's dynamic response occurred over the bat's behaviorally relevant range of ITDs. (Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference).

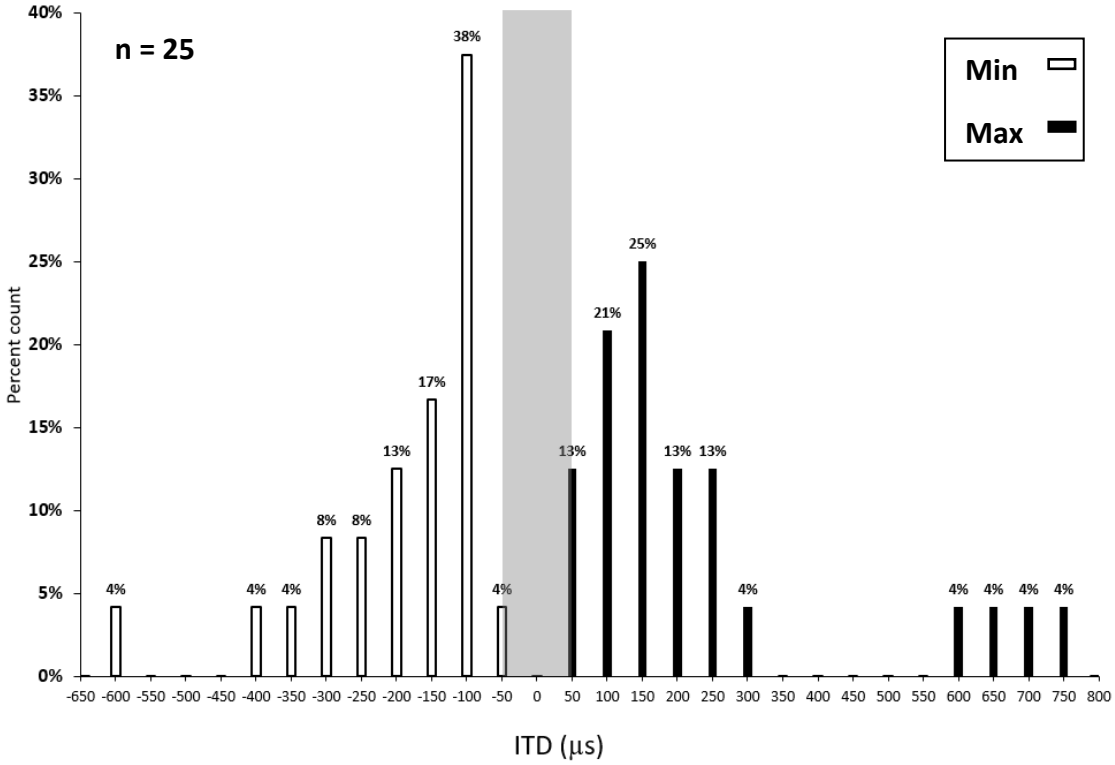

Fig. 7: Summary of minimum and maximum ITD responses for IC neurons whose ITD function passed through the behaviorally relevant range of ITDs (n=25). The response minimum (white bar) is the lowest response measured on a cell's ITD function, closest to the behaviorally relevant range (grey box; ± 50 µs). The response maximum (black bar) is the highest response measured on a cell's ITD function, closest to the behaviorally relevant range. The average minimum response across all cells was -224 ± 41.6 µs. The average maximum response across all cells was 236 ± 42.3 µs.

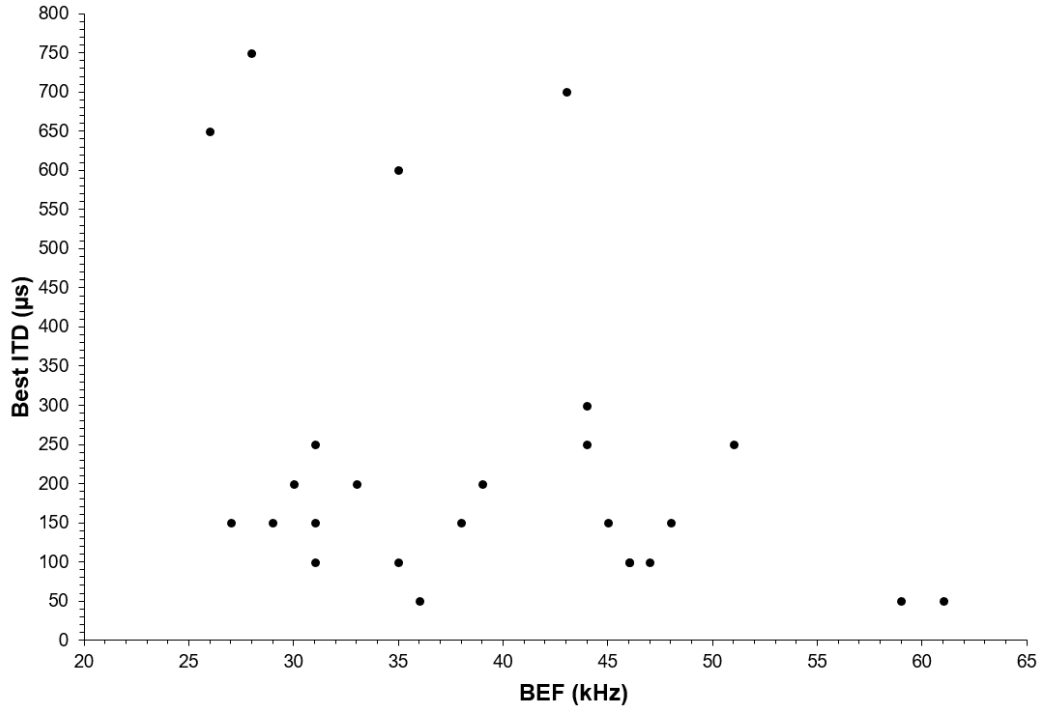

Fig 8: Distribution of best ITDs as a function of a BEF in the IC of *E. fuscus*. There was no correlation between a cell's BEF and its best ITD ($r = -0.351$, $n = 25$, $p = 0.086$).

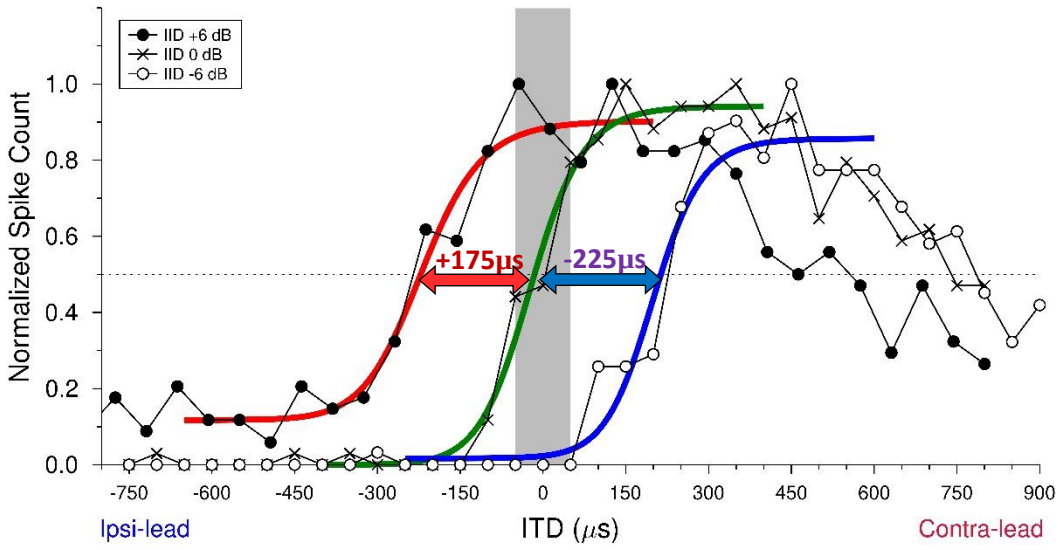

Fig 9: Visualization of time-intensity trading ratio calculation. A four-parameter sigmoid function (see Methods) was fitted to the ITD function for a cell at a particular IID (dashed lines). While holding the stimulus intensity in one ear constant, the stimulus intensity in the opposite ear was increased. An increase in the contralateral ear intensity resulted in positive IID values (blue sigmoid) whereas an increase in the ipsilateral ear intensity resulted in negative IID values. The arrows indicate the degree of shift caused by the change in intensity. The horizontal shift between two functions at their inflection point is measured across the ITD axis and divided by the change in stimulus intensity to obtain a time-intensity trading ratio. The inflection point at an IID of 0 dB (green sigmoid) was -25 µs. The inflection point at an IID of +6 dB (red sigmoid) was 200 µs. The inflection point at an IID of -6 dB (blue function) was 200 µs. The time-intensity trading ratio calculated between an IID of 0 dB and +6 dB was 29.2 µs/dB (||-25 µs – (-200) µs|| / 6 dB), whereas the trading ratio calculated between an IID of 0 dB and -6 dB was 37.5

μs/dB (||-25 μs – 200) μs|| / 6 dB). The average time-intensity trading ratio for this cell would thus be the average of all trading ratios calculated over a range of ITDs ((29.2 μs/dB + 37.5 μs/dB) / 2 = 33.4 μs/dB). Grey box represents the behaviorally relevant range of ITDs for *E. fuscus* (± 50 μs). Dotted line represents the 50% point of the neuron's maximum response. (BD = Best duration; Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference; IID = Interaural intensity difference).

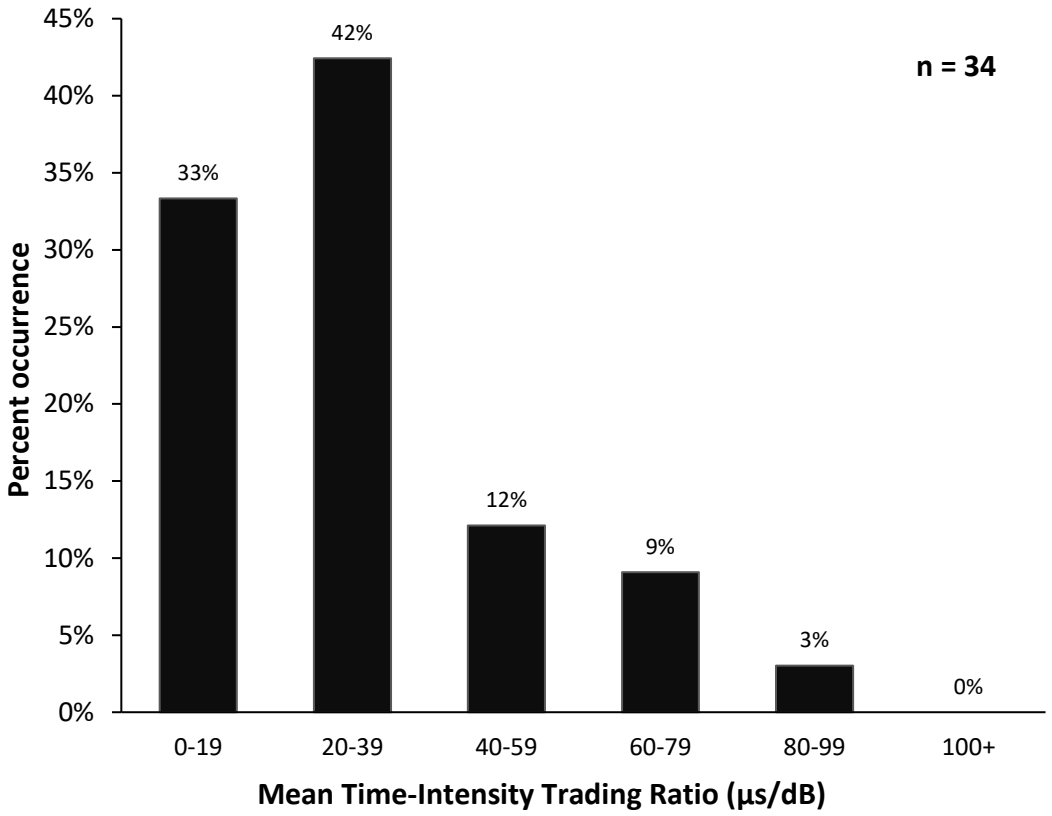

Fig 10: Distribution of time-intensity trading ratios calculated from 34 cells in the IC of *E. fuscus*. The average trading ratio measured across all cells was 30.2 ± 3.5 µs/dB.

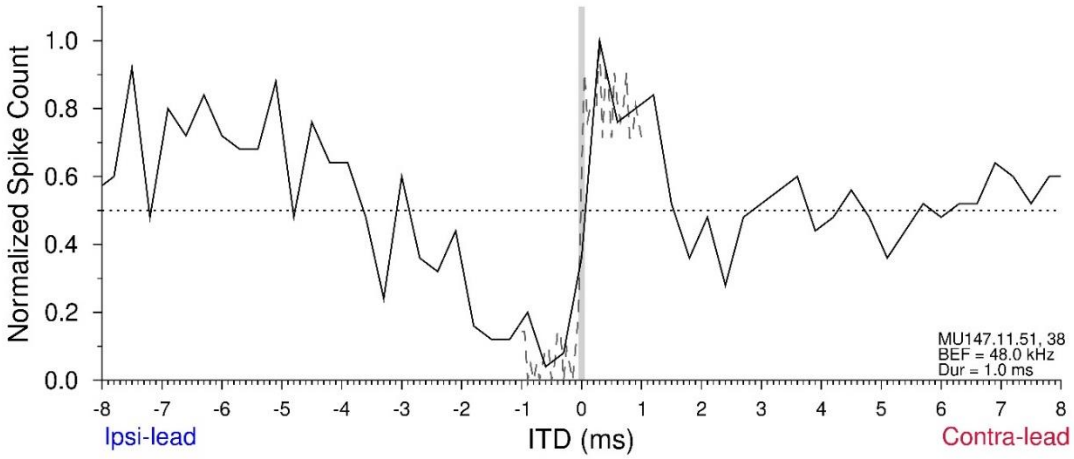

Fig 11: ITD response measured across a longer range of ITD values (± 8 ms) at a resolution of 300µs. Shown are normalized mean spike counts measured over 10-20 trials (error bars removed for clarity). Dotted line represents 50% of the maximum response. Grey box represents the behaviorally relevant range of ITDs for the bat (±50 µs). Dashed line represents the ITD function for the same cell measured at a finer resolution of 50 µs over ±1000 µs. (shown in Fig. 3A). (BEF = Best excitatory frequency; Dur = Stimulus duration; Ipsi = Ipsilateral; Contra = Contralateral; ITD = Interaural time difference).

Table 1

Summary of binaural response properties of IC neurons.

Binaural response type	N	Percent	Total tested
ITD-selective	52	65%	80
IID-selective	39	70.9%	55
Both IID and ITD selective	32	58.2%	55
Traded time for intensity	33 (among ITD-selective neurons)	63.5%	52
	22 (among IID-selective neurons)	56.4%	39

Table 2

Summary of IID response types of IC neurons.

IID Function Type	Binaural Response Type			
	EE (N=18)	EI (N=28)	EO (N=6)	Mixed (N=3)
Monotonic	None	22/28 (78.6%)	None	None
Peaked	5/18 (27.7%)	6/28 (21.4%)	None	1/3 (33.3%)
Non-selective	10/18 (55.6%)	None	6/6 (100%)	None
Reverse monotonic	2/18 (11.1%)	None	None	None
Mixed	1/18 (5.6%)	None	None	2/3 (66.6%)