BINAURAL RESPONSE PROPERTIES OF DURATION-TUNED NEURONS IN THE BAT

Frequency response of binaural inhibition underlying duration tuned neurons

by
Robert Mastroieni

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AUTHOR: Robert Mastroieni, B. Sc.

SUPERVISOR: Dr. Paul A. Faure

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Abstract

Auditory neurons selectively respond to frequency and amplitude of sound. In the auditory midbrain, duration-tuned neurons (DTNs) are subsets of neurons that selectively respond to the duration of sound. DTNs may help further understand the neural mechanism underlying temporal processing in the central nervous system. Temporal processing has been shown to play important roles in speech, discriminating species-specific signals, and echolocation. The goal of this thesis is to explore the role of DTNs through single-unit electrophysiological recordings in the auditory midbrain of the big brown bat (*Eptesicus fuscus*). Monotic and dichotic paired-tone stimulation was used to evoke excitatory and inhibitory responses from DTNs. Two stimuli consisted of best duration (BD) excitatory and non-excitatory (NE) tones. In the monotic condition, both tones were presented to the contralateral ear, and when they were close in time, the NE tone always suppressed spikes evoked by the BD tone. In the dichotic condition, the BD tone was presented to the contralateral ear. The NE tone was presented to the ipsilateral ear and suppressed BD tone evoked spiking in ~50% of cells. Properties of the ipsilaterally-evoked inhibition were investigated by varying the frequency of the NE tone from the best excitatory frequency (BEF), throughout a cell's excitatory bandwidth (eBW). We measured the inhibitory frequency response area, best inhibitory frequency (BIF), and inhibitory bandwidth (iBW) of each cell. We found inhibition became weaker as the frequency of the NE tone moved further from the middle of the eBW. We found that a DTN's BEF and BIF closely matched, but the eBW was broader than the iBW and overlapped the iBW measured from the same cell. This suggests temporal selectivity of midbrain DTNs are created by monaural inputs, with binaural inputs playing a lesser role in shaping duration selectivity.

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List of Abbreviations

BD - best duration
BEF - best excitatory frequency
BIF - best inhibitory frequency
BW – bandwidth
CNS – central nervous system
dB SPL - decibels sound pressure level
D_{BD} - duration of BD tone
D_{IHB} - duration of inhibition
D_{NE} - duration of NE tone
DTN - duration-tuned neuron
eBW - excitatory bandwidth
eFRA - excitatory frequency response area
FSL - first spike latency
H_{eBW} - high frequency partition of eBW
IC - inferior colliculus
ISI - interstimulus interval
L_{eBW} - low frequency partition of eBW
L_{first} - baseline first spike latency
L_{last} - baseline last spike latency
LSL - last spike latency
NE - non-excitatory
T_1 - onset of spike suppression
T_2 - offset of spike suppression
T_{end} - effective end time of inhibition
T_{start} – effective start time of inhibition

Introduction

Basic sounds can be described by three simple physical attributes: amplitude, frequency, and duration. The central nervous system (CNS) uses information about physical properties of sound to characterize acoustic signals in the surrounding environment. Through the use of physical and neural filters selective to these attributes, the central auditory system relays information to higher-level neural circuits that effect recognition, generate motor and sensory responses, and elicit behaviour (Chiel & Beer, 1997). Therefore, it is important to understand the brain circuits involved with characterizing basic features of sound.

Temporal properties of sound include frequency and amplitude modulations, signal onset and offset, interstimulus interval, stimulus ordering, and signal duration. Possibly the most basic and distinct of these acoustic features is stimulus duration. This feature is important in general functions such as the recognition of human speech (Denes, 1995), distinguishing conspecific social and mating calls (Narins & Capranica, 1978; Pollack & Hoy, 1979), and discriminating sounds generated by predators and/or prey (Ehrlich *et al*., 1997; Faure & Hoy, 2000). Stimulus duration is especially important in specialized auditory behaviours such as echolocation in dolphins and bats (Au & Benoit-Bird, 2003; O'Neill & Suga, 1982). Most vertebrates rely on signal duration information as part of normal hearing, which emphasizes the importance of encoding, and signal detection; however, compared to studies examining the encoding of stimulus frequency and/or amplitude, relatively less is known about how the brain encodes the duration of an acoustic stimulus.

Duration-tuned neurons (DTNs) are central auditory cells that exhibit neurophysiological responses that are selective to specific stimulus durations. In echolocating bats, responses of these cells are selective for signal durations on the order of milliseconds, and DTNs also respond to specific signal frequencies and amplitudes (Morrison *et al*., 2014). Duration-tuned neurons are found in a large number of vertebrate species at varying regions

of the central auditory pathway at and above the inferior colliculus (IC) in bats (Ehrlich *et al*., 1997; Faure *et al.*, 2003), chinchillas, (Chen, 1998), cats (He *et al*., 1997), rats (Pérez-González *et al*., 2006), mice (Xia *et al*., 2000) and guinea pigs (Wang *et al*., 2006). First discovered from the torus semi-circularis (auditory midbrain) of frogs (Potter, 1965; Gooler & Feng, 1992), DTNs have also been reported from the auditory thalamus in guinea pigs (He, 2002), and the auditory cortex of both cats (He *et al.*, 1997) and bats (Galazyuk & Feng 1997). Moreover, DTNs have been reported from the visual cortex of cats (Duysens *et al.*, 1996) and possibly from the peripheral nervous system of mormyrid electric fish (Lyons-Warren *et al*., 2012). Across all these examples, the responses of DTNs appear to encode signal durations within ranges relevant to each species depending on the ecology of the organism (Sayegh *et al*., 2011). This suggests an inherent value of DTNs in the vertebrate nervous system, as they have been found in different taxa.

A cell's best excitatory frequency (BEF) is defined as the frequency of the stimulus that evoked the maximum spike count. A cell's excitatory bandwidth is determined by stimulating a neuron with a constant amplitude, varying frequency, pure tone that was set to durations of 1ms and 4ms. A cell's best duration (BD) is defined as the duration of the stimulus that evokes the maximum spike count. To determine the BD, a neuron is stimulated with constant amplitude, variable duration, pure-tone pulses presented at the cell's preliminary BEF captured in the previous phase. The final BEF was determined with threshold amplitude, and the previously determined BD (Sayegh *et al.,* 2014).

There are at least four different types of DTNs whose response classes are defined based on the number and the pattern of spiking evoked across different stimulus durations (Faure *et al.*, 2003). (1) Short-pass DTNs respond most to sounds presented at the BD of the cell, with a reduction of spiking (≥50%) to signals above that duration. (2) Band-pass DTNs also respond maximally to their BD, but exhibit a ≥50% reduction in spiking at signal durations above and below the BD (Fig. 1). (3) Long-pass DTNs do not have a BD because

the cell responds to any stimulus above some minimum duration. These cells exhibit a ≥50% reduction in spiking at stimulus durations shorter than this minimum duration. (4) Band-reject DTNs are complex as they are characterized as having at least one BD peak with an additional response peak. Between the peaks a ≥50% reduction in spiking may occur (Mora & Kössl 2004). A potential variant of bandreject DTNs are multi-peak DTNs, characterized as DTNs with multiple response peaks including a BD with minimal response in-between the peaks.

Mechanistically, duration tuning in the auditory pathway is thought to be created at and above the level of the auditory midbrain by the temporal interaction of neuronal excitation and inhibition. Experiments involving electrophysiology (Faure *et al.*, 2003), neuropharmacology (Casseday *et al*., 1994) and computational data (Aubie *et al.*, 2009) support this hypotheses. Conceptual models created from known intracellular and extracellular response properties can reproduce the spiking patterns of *in vivo* DTNs recorded in multiple vertebrate species (Aubie *et al*., 2012). The exact roles of duration tuning and DTNs in vertebrate hearing are still unknown. It has been hypothesized that duration tuning may be important for echolocation by bats. For example, in the big brown (*Eptesicus fuscus*), velvety free-tailed (*Molossus molossus*), and mustached bat (*Pteronotus parnellii*) it has been found that the BDs of many DTNs appear to be well-matched to the range of call durations emitted by the bats while hunting (Ehrlich *et al*., 1997; Mora & Kössl, 2004; Macias *et al.*, 2013). In other mammals that do not echolocate, DTNs are thought to play a role in stimulus identification (Sayegh *et al*., 2011).

The monaural paired-tone stimulation paradigm has been used to characterize sound evoked inhibition in DTNs (Faure *et al*., 2003). This paradigm involves the presentation of two tones, a BD tone and a second test tone of the same frequency but presented at a nonexcitatory (NE) duration. The onset time of the NE tone is fixed, while onset time of the BD tone is varied. As the BD tone is moved relative to the NE tone, the interaction between the two signals results in spike suppression when excitation evoked by the BD tone overlaps

with inhibition evoked by the NE tone. The time course of the spike suppression is a measure of the duration of inhibition evoked by the NE tone. A few phenomena have been used previously to characterize the strength and time course of inhibitory inputs to DTNs. If the latency of the inhibition was shorter than the excitatory first spike latency, the cell was said to have leading inhibition. If the latency of the inhibition was longer than the excitatory first spike latency, the cell was said to have lagging inhibition. When the monaural inhibition lasts as long as the duration of the NE tone, it was defined as sustained inhibition. Inhibition that lasted longer than the duration of the NE tone was characterized as persistent inhibition.

Valdizón-Rodríguez and Faure (2017) investigated the properties of monaural inhibition in DTNs by varying the NE tone frequency in paired-tone stimulation. They collected spike count recordings when varying the frequency of the NE tone away from the BEF, both within and outside the excitatory bandwidth of the cell. The spikes evoked by the BD tone became suppressed by an onset-evoked, inhibitory input. The duration of the spike suppression decreased as the NE tone frequency moved further away from the cell's BEF, likely because the duration of the inhibition evoked by the NE tone also decreased. The authors concluded that DTNs receive an onset-evoked, constant-latency, inhibitory input within the excitatory receptive field of a cell. Additionally, the offset and duration of the inhibition decreased as the stimulus departed from the cell's BEF. Finally, the authors found that the best inhibitory frequency matched the best excitatory frequency; however, a cell's inhibitory bandwidth was more broadly tuned than excitatory bandwidth.

Most studies on auditory DTNs have been conducted with monaural acoustic stimulation. The effects of binaural stimulation on the response properties of DTNs have not been systematically explored. A binaural paired-tone stimulation paradigm is similar to the monaural paired-tone stimulation paradigm; the BD tone is still presented to the contralateral (i.e. excitatory) ear but the NE tone is presented to the ipsilateral (i.e. inhibitory) ear relative to the IC being recorded. Sayegh *et al.*, (2014) used both monotic and dichotic paired-tone

stimulation to measure the relative contributions of the contralateral and ipsilateral neural circuits in duration tuning. They found that all DTNs receive monaural inhibition evoked by stimulation of the contralateral ear would always occur when the NE tone closely preceded or followed the excitatory, BD tone in the contralateral ear. In the dichotic condition, spikes evoked by the BD tone presented to the contralateral ear were suppressed in only half of the cells tested when the NE tone was presented to the ipsilateral ear. Additionally, when DTNs received ipsilaterally-evoked inhibition this inhibition was weaker in strength, longer in latency, and shorter in duration compared to the monaural inhibition evoked from the contralateral ear.

The present study aims to further characterize the response properties and frequency tuning of the ipsilaterally-evoked synaptic inhibition acting on DTNs in the mammalian inferior colliculus using binaural paired-tone stimulation. Stimulus frequency has been found to affect the interaction of excitatory and inhibitory synaptic inputs, as the duration of the sustained inhibition acting on DTNs shortened, relative to that evoked at the BEF, as the NE tone frequency moved further from the BEF (Valdizon- Rodriguez & Faure, 2017). Understanding the spectral tuning of the ipsilaterally evoked inhibitory inputs to DTNs will provide greater insight into the neural mechanism and functions of duration selectivity in the mammalian central auditory system at non-characteristic frequencies.

Materials and Methods

Surgical preparation

Electrophysiological recordings were obtained from the IC of 22 big brown bats (*Eptesicus fuscus;* 21 males, 1 female). Prior to recording, each bat underwent a preparatory surgery that resulted in the gluing of a small stainless steel post to the dorsal surface of the skull immediately rostral to the IC. The headpost prevented movement of the bat's head during recording and allowed me to accurately replicate the bat's head position between

recording sessions and subjects in a stereotaxic apparatus (David Kopf Instruments model 1900). Before surgery, bats were administered buprenorphine (~0.03 mL SQ temgesic; 0.025 mg/kg). Following injection, the bats were placed in an anesthesia induction chamber (12 x 10 x 10 cm) where they inhaled a 1-5% isoflurane:oxygen (flow rate = 1 L/min). Once anaesthetized, bats were then placed in a foam-lined body restraint, which holds the bat firmly but comfortably while still allowing access to its head. To maintain anesthesia and stabilize the head during surgery, the bat's mouth was placed in a custom bite bar fitted as a gas mask for the delivery of anesthetic. The hair overlying the skull was then shaved and the skin was disinfected with a povidone-iodine surgical scrub (Betadine®). Prior to making a midline incision in the scalp, a local anesthetic (~0.2 mL Bupivicaine SQ, 5 mg/mL) was administered. The temporal muscles were then reflected and the dural surface of the skull was scraped clean and swabbed with 70-100% ethanol. A metal head post was then glued to the dorsal surface of the skull using cyanoacrylate (Superglue) adhesive cured with liquid hardener (Pacer Zipkicker). One end of a chlorided silver wire attached to the head post was placed under the temporal musculature and served as the reference electrode. A piece of Gelfoam® coated with Polysporin® covered the wound to prevent infection. Following surgery, bats recovered and were housed individually in stainless steel holding cages (28 x 21 x 21; 1/4 - inch mesh). Cages were located in a temperature and humidity controlled holding room, with food and water provided *ad libidum*. All procedures were approved by the McMaster University Animal Research Ethics Board and were in accordance with guidelines for the care and use of experimental animals published by the Canadian Council on Animal Care.

Electrophysiological recordings

Recordings began 1-3 days following surgery. Bats were used in 6-8 recording sessions, lasting 6-8 hours each on different days. Sessions would be aborted if the bat

displayed signs of discomfort. After a recording session, the wound was covered with a piece of contact lens and Gelfoam® coated in Polysporin®. Recording sessions were held inside a double-walled, sound attenuation booth with electrical shielding (Industrial Acoustics Co., Inc.). Prior to recording, bats were administered a neuroleptic (0.3 mL of a 1:1 v/v mixture of 0.025 mg/mL fentanyl citrate and 1.25 mg/mL Inapsine [droperidol]; 19.1 mg/kg or 0.4 mL of a 5:1 v/v mixture of 0.4 mg/mL fentanyl citrate and 2 mg/mL midazolam; 1 mg/kg). After the neuroleptic took effect, bats were placed in a foam-lined body restraint that was suspended by springs within a stereotaxic frame customized for bats (ASI Instruments). The entire apparatus was placed atop an air vibration table (TMC Micro-G). To immobilize the bats, the head post was secured to a stainless-steel rod attached to a manual micromanipulator (ASI instruments) mounted on the stereotaxic frame (David Kopf Instruments). To access the IC for recording, a craniotomy was performed with a scalpel blade and the dura mater was then removed with a sharp pin. The skull of the bat is translucent and the ICs can be visually identified as two white ellipses caudal to the cortex. Single-unit extracellular recordings were obtained with thin-walled, borosilicate glass microelectrodes (outside diameter = 1.2 mm; A-M Systems, Inc.) filled with 1.5 M NaCl. Typical electrode resistances ranged from 15 - 30 MΩ. Electrodes were positioned over the exposed IC, using manual micromanipulators (ASI Instruments) and lowered into the brain with a stepping hydraulic micropositioner (Kopf model 2650). A neuroprobe amplifier (A-M Systems model 1600) whose 10x output was band-pass filtered and further amplified (500-1000x) by a Tucker Davis Technologies spike pre-conditioner (TDT PC1; lowpass f_c = 7 kHz; high-pass f_c = 300 Hz) was used to record extracellular action potentials. Spike times were logged onto a computer by passing the TDT PC1 output to a spike discriminator (TDT SD1) and then an event time (TDT ET1) synchronized to a timing generator (TDT TG6).

Stimulus generation and data collection

Stimulus generation and online data collection were controlled with custom software that displayed spike-times as a dot raster displays sorted by the acoustic parameter that was varied. Auditory stimuli were digitally generated with 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 anti-aliasing filter (TDT FT6-2; f_c = 120 kHz), programmable attenuator (TDT PA5), and two signal mixers (TDT SM5) with equal channel weighting. The output of each mixer was sent to a manual attenuator (Leader LAT-45) prior to final amplification (Krohn-Hite model 7500). Acoustic stimuli were broadcast with Brüel & Kjær (B&K) ¼ inch condenser microphones (Type 4939; protective grid on) modified for use as loudspeakers with transmitting adaptors (B&K Type UA-9020) to correct for nonlinearities in their transfer functions. Each loudspeaker was positioned ~1 mm in front of the bat's external auditory meatus. The output of each speaker was recorded with a B&K Type 4138 ⅛-inch condenser microphone (90° incidence; grid off) connected to a measuring amplifier (B&K Type 2606), band-pass filter (Krone-Hite model 3500) and sound calibrator (B&K Type 4231) and was expressed in decibels sound pressure level (dB SPL re 20 µPa) equal to the peak amplitude of continuous tones of the same carrier frequency (Frederiksen, 1977). The loudspeaker transfer functions were flat ±6 dB from 28–118 kHz, and there was at least 30 dB attenuation at the ear opposite of the source (Ehrlich *et al.* 1997). At low sound frequencies the transducers generate harmonic distortions hence we excluded data points collected with frequencies <15 kHz. All acoustic stimulation had rise/fall times of 0.4 ms, shaped with a squared cosine function, and were presented at a rate of 3 Hz.

Search stimuli were composed of two pure tones that differed only in duration (1 and 4 ms; ISI ≥ 110 ms) and were presented monaurally to the ear contralateral to the IC recorded. Once a cell was isolated, we determined its BEF (0.1-1 kHz resolution), BD (1 ms resolution),

minimum acoustic threshold (5 dB resolution), and duration filter response class at the BEF (Sayegh *et al*., 2011).

Using each cell's BEF, BD and minimum acoustic threshold, a cell's excitatory frequency response area (eFRA) was measured at +10 dB re threshold to determine its excitatory spectral bandwidth (eBW). The eBW was defined as the frequency range (maximum – minimum) where the spike count fell to = 50% of the maximum count evoked at the BEF. The sharpness of excitatory tuning, measured with paired-tone stimulation at + 10 dB (re BD, BEF threshold), was calculated as a Q factor defined as Q_{10db} = BEF/eBW. Paired-tone stimulation allowed us to measure an inhibitory Q factor (Q = BIF/iBW), but unlike our excitatory $Q_{10\ dB}$ the level above the inhibitory threshold at which the inhibitory Q was measured was unknown.

Paired tone stimulation with BEF and non-BEF NE tones

We used the stimulus paradigms for monaural and binaural paired-tone stimulation as described by Faure *et al.* (2003) and Sayegh *et al.* (2014), respectively.

Monaural paired-tone stimulation was used to measure the strength and time course of the contralateral inhibition evoked by a longer duration, non-excitatory (NE) tone. In the dichotic condition this NE tone was varied in frequency (see Valdizon- Rodriguez & Faure, 2017). The basic paradigm involves stimulating a cell with two pure tone pulses that initially only differ in duration and interstimulus interval (ISI) (Faure *et al.*, 2003). The first tone, the BD tone, was set to the cell's BD and BEF to evoke maximum excitatory spiking. The second tone, the NE tone, was set to a non-excitatory duration that was typically ten times the duration relative to the BD tone to ensure a constant energy relationship between the two signals, regardless of the cell's BD. The onset time of the NE tone was fixed between stimulus presentations, whereas the onset time of the BD tone was randomly varied in time (2 - 4 ms steps) so that it occurred before, during, and after the NE tone. The pair of tones were

electronically mixed and presented to the contralateral ear at an amplitude of +10 dB (re BD tone threshold). The signals were phase matched hence when the two tones were the same frequency they would always constructively interfere causing a composite tone with an amplitude pedestal of +6 dB for the duration of stimulus overlap. When the tones were not matched in frequency, the resulting composite signal would contain an amplitude pedestal that was sinusoidally amplitude modulated with a modulation index = 1 and a modulation frequency equal to the difference between the two stimulus frequencies.

Binaural (dichotic) paired-tone stimulation was used to measure the strength and time course of ipsilaterally-evoked inhibition at varying NE tone frequencies. Binaural paired-tone stimulation was implemented once a cell's BEF, BD, acoustic threshold, and monaural paired-toned stimulation responses were collected. In this paradigm, two tones were presented from different microphones. The first tone, the BD tone, was set to the cell's BEF, BD and +10 dB above the minimum threshold so that reliable spiking could be evoked to measure a baseline response. The second tone, the NE tone, was set to the cell's BEF and was also presented at +10 dB above threshold; however the duration was set to a non-excitatory tone (10x the BD). The onset time of the NE tone was fixed whereas the onset time of the BD tone was randomly varied. The BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear. This inhibitory tone was used to identify changes in spiking responses, as any changes in spiking were caused by the NE tone presented to the ipsilateral ear.

We tested DTNs with dichotic, binaural paired-tone stimulation at five NE tone frequencies standardized to the eBW of each cell (Fig. 2). To obtain the standard frequencies, the eBW was divided into lower (L_{eBW}) and higher (H_{eBW}) spectral partitions (re BEF). The five standardized frequencies were selected as: (1) 1.5 times below the L_{eBW} below the BEF ($1.5L_{eBW}$); (2) the midpoint of the L_{eBW} ($0.5L_{eBW}$); (3) the BEF; (4) the midpoint of the H_{eBW} ($0.5H_{eBW}$); and (5) 1.5 times above the H_{eBW} above the BEF ($1.5H_{eBW}$). Thus, each cell was

tested with at least three NE tone frequencies within its 50% eBW (BEF, $0.5L_{eBW}$; $0.5H_{eBW}$) and two NE tone frequencies outside its 50% eBW ($1.5L_{eBW}$, $1.5H_{eBW}$). Whenever possible, additional NE tone frequencies were also tested. Using monaural and binaural paired-tone stimulation, we measured and compare the inhibition evoked by the NE tone through the monaural (contralateral) and binaural (ipsilateral) central auditory pathways.

Measuring NE tone evoked inhibition with spike counts and latencies

The latency and duration of the inhibition evoked by the NE tone was quantified by determining the ISIs that showed significant deviations in the BD tone evoked spike count and/or latencies used was the same criterion established by Sayegh *et al.* (2014). In both monotic and dichotic paired-tone stimulation, a cell's baseline responses were quantified at the ten longest ISIs when the BD tone occurred prior to the NE tone (see Fig. 3) to ensure that the cell's responses were not affected by NE tone-evoked inhibition. The mean ± standard deviation (SD) baseline spike count, first spike latency (FSL), and last spike latency (LSL) were calculated for each cell with these responses.

Using three criteria, we compared baseline responses with those obtained at other ISIs to determine the points in time spike counts or latencies were suppressed and/or altered in latency by NE tone-evoked inhibition. A 50% change in the evoked spike count (re baseline) was the initial criterion used to determine the time points demarking the onset and offset of spike suppression. This criterion is the same as previous paired-tone stimulation studies (Faure *et al.*, 2001; Sayegh *et al.*, 2014; Valdizon-Rodriguez & Faure, 2017). When possible, a 1 SD change in the evoked spike latency (re baseline) was used to refine and extend these estimates because changes in spike latency are a more sensitive measure of inhibitory effects compared to changes in spike count.

Each criterion yielded a set of ISIs, T_1 and T_2, when BD tone evoked spikes were suppressed and/or altered in latency. From these we determined the onset, and offset of the

altered response. The onset of spike suppression (T_1) was defined as the shortest ISI, starting from when the BD tone preceded the NE tone and moving towards larger positive ISI's, when the spike count and/or latency became altered and the following two consecutive ISI's were also altered for a given criterion. The offset of spike suppression (T_2) was defined as the shortest ISI, following T_1, when the BD tone evoked spike count and/or latency remained altered and the next two consecutive ISI's had returned to within baseline values for a given criterion.

The final values for T_1 and T_2 were chosen using the most sensitive criteria reflecting the time course of spike suppressions evoked by the NE tone using changes in the BD tone evoked spike count and/or spike latency. Utilizing changes in a cell's spike counts and/or latencies to quantify changes in neuronal responsiveness has been previously used to measure inhibition in DTNs (Faure *et al*., 2003; Sayegh *et al*., 2014; Valdizon-Rodriguez & Faure, 2017). In cases where a cell responded with only a single spike per stimulus (i.e. $L_{first} = L_{last}$), a change in spike count was typically used for selecting T_1 and T_2 because this criterion was more sensitive in reflecting the time course of the NE tone evoked inhibition. For cells that responded with more than one spike per stimulus (i.e. $L_{first} < L_{last}$) or when the spike count of a cell had recovered to within 50% of baseline even though L_{first} or L_{last} (or both) were still clearly deviated by >1 SD from baseline, a change in spike latency was typically used for selecting T_1 and T_2 because this criterion was more sensitive in reflecting the time course of the NE tone evoked inhibition. In cases where the mean spike count or latency had not returned to within 50% or 1 SD of baseline, respectively, over the range of ISI's presented, T_2 was conservatively estimated as the longest ISI tested.

Similar to previous studies (Faure *et al*. 2003; Sayegh *et al*., 2014; Valdizon-Rodriguez & Faure, 2017), a mixture criteria, either a 50% change in spike count or a 1 SD change in spike latency, was used to determine the onset/offset of altered responses in DTNs during paired-tone stimulation. This method is sensitive and allows for an accurate measure of

the time course of NE tone-evoked inhibition during monaural and binaural paired tone stimulation. Initially, spike counts were the criterion used, and spike latencies were used when possible to extend the estimate for duration of inhibition.

Calculating the latency and duration of NE tone evoked inhibition

After determining the values of T_1 and T_2, we calculate the effective start time (T_{start}), end time (T_{end}) and duration of inhibition (D_{IHB}) evoked by the NE tone using the following formulas:

$$T_{start} = T_1 + L_{last} - D_{BD} \quad (1)$$

$$T_{end} = T_2 + L_{first} - D_{BD} \quad (2)$$

$$D_{IHB} = T_{end} - T_{start} \quad (3)$$

where L_{last} was the baseline LSL, L_{first} was the baseline FSL, and D_{BD} was the duration of the BD tone. The onset (T_1) and offset (T_2) of changes in a cell's evoked responses were detected at ISIs where the BD tone-evoked spike count and/or latency deviated. Because the paired-tone stimulation paradigm uses a roving BD tone and a stationary NE tone, the changes that occur in spiking depends on the timing of the two tones. When the BD tone leads (follows) the NE tone, it is the last (first) spikes in the BD tone-evoked response that initially (finally) become altered (recovered) in number owing to the onset (offset) of inhibition evoked by the NE tone. Therefore, in the equation for T_{start} (T_{end}) the baseline LSL (FSL) is added to T_1 (T_2) because spikes would have occurred at this point in time were they not suppressed by inhibition evoked by the NE tone. Since T_1 (T_2) was measured with respect to BD tone offset whereas the baseline LSL (FSL) was measured with respect to BD tone onset, subtracting the duration of the BD tone from T_1 + LSL (T_2 + FSL) aligns both time axes with respect to NE tone onset.

A neuron was said to have leading inhibition when the latency of the inhibition evoked by the NE tone was shorter than the cell's excitatory FSL (i.e., $T_{start} < L_{first}$), but was

said to have lagging inhibition when the onset of NE tone evoked inhibition occurred after the FSL (i.e. $T_{start}>L_{first}$). A neuron was said to have persistent inhibition when the effective duration of the inhibition (D_{IHB}) evoked by the NE tone was greater than the duration of the NE tone stimulus (i.e. $D_{IHB} > D_{NE}$).

Figure 3 depicts example responses from a DTN tested with monaural paired-tone stimulation to illustrate how we calculated baseline response properties and measured the start (T_{start}), end (T_{end}) and effective duration of inhibition (D_{IHB}) evoked by the NE tone. The cell illustrated had responses with T_{start} = 19.25 ms, T_{end} = 57.66 ms, and D_{IHB} = 38.41 ms. Leading inhibition was present because the latency of inhibition evoked by the NE tone occurred 2.41 ms before the 21.66 ms FSL (L_{first} - T_{start} = 2.41 ms). The neuron had persistent inhibition because the duration of the inhibition evoked by the NE tone was 8.41 ms longer than the duration of the 30 ms NE tone (D_{IHB} - D_{NE} = 8.41 ms). Following stimulation, the mean spike count at the 10 longest positive ISIs (1.16 ± 0.83 spikes/stimulus) was still significantly lower [$t(15.7) = 5.6$, $p << 0.01$] than the cell's baseline spike count (1.76 ± 0.80 spikes/stimulus). This means that the cell's evoked spike count had still not fully recovered to its baseline levels. In contrast, the cell's FSL and LSL had both recovered and were not significantly different from the cell's baseline FSL (21.66 ms) or LSL (25.25 ms). The FSL and LSL values returned to baseline after stimulation because the evoked inhibition from the NE tone ended.

Calculating the iFRA, BIF and iBW

To measure the best inhibitory frequency (BIF) and inhibitory bandwidth (iBW) of a DTN at a + 10 dB (re threshold, we first had to obtain a cell's normalized iso-level inhibitory frequency response area (iFRA). This was done by dividing the duration of inhibition evoked at each NE tone frequency by the duration of inhibition evoked at the BEF, and plotting as a function of the NE tone frequency in octaves re BEF (Fig. 4). We also calculated the

difference in octaves between $\log_2$(BIF) - $\log_2$(BEF) so that we could compare the BEF with the BIF for each cell. The BIF of a DTN was the frequency with the largest normalized D_{IHB}. The iBW was calculated with separate linear regressions measured from the slopes of the iFRA tuning curve. The low ($Slope_{Low}$) and high ($Slope_{High}$) frequency slopes of a cell's inhibitory tuning curve (re BIF) were used to define the cutoff frequencies through interpolating each regression to 50% at the value of the BIF. For these regressions, the highest and lowest frequencies included were the first normalized data points ≤0.1, starting from the BIF and moving either higher or lower in frequency. A quality (Q) factor, defined as Q = BIF/iBW, was calculated to determine the sharpness of inhibitory tuning during paired-tone stimulation at +10 dB (re BD, BEF threshold) and was compared to sharpness of excitatory tuning calculated from each cell's eFRA (i.e. Q_{10dB} = BEF/eBW).

Data Analysis

All data are reported as the mean ± standard error (SE). Data were tested for normality and homogeneity of variances with Shapiro-Wilk's and Bartlett tests, respectively, before additional statistical analyses were performed. Parametric tests were used when the data were normally distributed with equal variances; otherwise, equivalent nonparametric tests were used. A total of 38 DTNs were recorded from the IC of *E. fuscus*. Unfortunately, the five standardized points were not always obtained from every cell (e.g. a cell was lost), and this resulted in a decreased sample size. Some DTNs did not display measurable inhibition during binaural paired-tone stimulation at each of the 5 standardized NE tone frequencies, and when this occurred the data from these cells were excluded from repeated-measures statistical analyses; however, the data were included in summary statistics and figures and/or tables.

A Kruskal-Wallis test was used to compare the proportion of DTNs that exhibited leading and persistent inhibition at each NE tone frequency. A chi-squared test was used to

determine differences in leading and persistent inhibition across NE tone frequencies in the dichotic condition. Mann-Whitney *U* tests were used to compare the duration of leading inhibition between short-pass and band-pass DTNs. The latency and time course of the inhibition evoked in monaural and binaural paired-tone stimulation were compared with paired *t*-tests or Wilcoxon's signed-rank tests. A Wilcoxon signed-rank test was used to compare eBWs and iBWs, excitatory and inhibitory Q factors, and the $Slope_{Low}$ and $Slope_{High}$ values measured from each cell's iFRA. Linear regressions were used to calculate the relation of BD, FSL, and leading/lagging inhibition at the 5 standardized NE tone frequencies and to measure the low ($Slope_{Low}$)- and high ($Slope_{High}$)-frequency tuning slopes of the iFRA for each cell. All statistical tests were performed in SPSS and used and experiment-wise error rate of $\alpha = 0.05$.

Results

Inhibition evoked with monotic and dichotic paired-tone stimulation

Figure 5 shows a dot raster display of a short-pass DTN that was tested with both monotic (5A) and dichotic (5E) paired-tone stimulation with the BD and NE tones set to the cell's BEF with both tones at equal amplitudes. In the monotic condition, the cell displayed a longer duration of inhibition (40.22 ms) when compared to the dichotic condition (32.60 ms). There was a significant suppression in the cell's evoked spike counts (Fig. 5B) and deviations in both the FSL and LSL (Fig. 5, C and D). The final value of T_1 was 2 ms and the final value of T_2 was 46 ms; and these were determined from deviations in LSL and spike counts respectively. The DTN displayed lagging inhibition, because $L_{First} = 8.01$ and $T_{start} = 9.79$ ms, hence the inhibition occurred 1.78 ms following the cell's excitatory FSL. There was a small amount of persistent inhibition because the effective duration of inhibition ended 0.22 ms after the offset of the 40-ms NE tone.

In the dichotic condition (Fig. 5E), the BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear. In the binaural (dichotic) condition, there was a noticeable decrease in the strength and duration of the NE tone evoked inhibition. There was a reduction in the cell's spike count (Fig. 5F) and there were significant deviations in the FSL when the 4-ms BD tone and 40-ms NE tone overlapped in time (Fig. 5G). It should be noted that no significant deviation in the cell's LSL was detected (Fig. 5H). The cell's average baseline spike count, determined at the first 10 ISIs, was 1.56 ± 0.75 spikes/stimulus compared to 0.05 ± 0.62 spikes/stimulus calculated during the presentation of the NE tone resulting in inhibition. The final value of T_1 was 8 ms, and the final value of T_2 was 44 ms, and both were obtained using changes in the cell's spike count. The duration of spike suppression was 32.60 ms, with the neuron displaying lagging inhibition because L_{first} = 7.66 and T_{start} = 15.05 ms; therefore, inhibition occurred 7.40 ms following the cell's FSL. There was no evidence of persistent inhibition in this condition because the effective duration of inhibition ended 7.40 ms before the offset of the 40-ms NE tone.

Inhibition evoked with dichotic paired-tone stimulation at the BEF and at non-BEFs

Figure 6 shows a dot raster display of the same DTN in Figure 5, now tested with dichotic paired tone stimulation with the BD tone set to the cell's BEF and the NE tone set to the BEF (A) and to a non-BEF (E) and with both tones at equal amplitudes. This cell displayed weak spike suppression when the ipsilateral NE tone was at the cell's BEF, and no measurable inhibition when the ipsilateral NE tone was not at the BEF. When stimulated at +10 dB re threshold, the BEF of this cell was 49 kHz. As described previously in Fig. 5B, the DTN exhibited weak inhibition in the dichotic condition (re to the monotic condition) when the NE tone was set to the cell's BEF. Changes in the cell's spike count (Fig. 6B) and deviations in its FSL (Fig. 6C) were shown during indicating spike suppression. There was no measurable inhibition when looking exclusively at changes in the cell's LSL (Fig. 6D).

The following is an example of when the NE tone was set to a frequency within the 50% eBW but not at BEF. In the non-BEF condition, the BD tone presented at BEF (49 kHz) differed from the NE tone (52 kHz, $0.5H_{eBW}$) and this resulted in an absence of measurable spike suppression (Fig. 6E). Throughout all ISIs, the spike count never dropped to ≤ 50% of the baseline spike count (Fig. 6F), and both the FSL and LSL never deviated by >1 SD from the baseline values, respectively, over 2 successive ISIs (Fig. 6, G and H).

The following is an example of when the NE tone was set to a frequency outside of the 50% eBW. Figure 7 shows a dot raster display of a short-pass DTN that was tested with dichotic paired-tone stimulation when the NE tone was set to the cell's BEF (7A) and non-BEF (7E). The cell displayed inhibition when the ipsilateral NE tone was at the cell's BEF, and weaker spike suppression when the ipsilateral NE tone was presented at a non-BEF outside of the 50% eBW, When stimulated at +10 dB re threshold, the BEF of this cell was 34 kHz. At BEF, the baseline spike count, determined at the first 10 ISIs, was 1.82 ± 0.74 spikes/stimulus compared to the 0.06 ± 0.11 spikes/stimulus displayed during the ongoing portion of the NE tone, whose offset was not captured in the raster display as additional tests at longer ISIs were not collected. Reductions in spike count (Fig. 7B) and significant deviations the FSL (Fig. 7*C*) were exhibited as well when the 5-ms BD tone and 50-ms NE tone were close in time. There were no deviations for the LSL for this cell (Fig. 7*D*). The final value of T_1 was 1 ms, and was derived from either changes to the cell's spike count or FSL. The final value for T_2 was 75 ms and was derived from changes in the cell's spike count. The duration of spike suppression was 64.57 ms, with the neuron displaying leading inhibition because L_{first} = 16.49 and T_{start} = 13.92 ms; therefore the onset of inhibition occurred 2.57 ms prior to the cell's FSL. Additionally, there was a prominent amount of persistent inhibition because the effective duration of inhibition ended 14.57 ms beyond the offset of the 50-ms NE tone.

Unlike the cell in Figure 6, when the frequency of the stationary NE tone was set to a non-BEF of 43 kHz and was outside the cell's 50% eFRA($1.5H_{eBW}$), the cell in Figure 7E exhibited spike suppression albeit for a shorter duration. Compared to the BEF condition, the T_1 value increased to 9 ms and the final value of T_2 decreased to 27 ms. Both were derived from a spike count criterion (Fig.7F). With regard to spike latency changes there were no deviations > 1 SD from baseline over 2 consecutive ISIs for either the FSL and/or LSL (Fig. 7*G* and *F*). Changing the frequency of the NE tone away from the BEF resulted in a shortened duration of suppression to 17.61 ms. Lagging inhibition occurred in this condition because L_{first} = 17.85 and T_{start} = 22.24 ms; therefore inhibition occurred 4.39 ms following the excitatory FSL. Additionally, there was no evidence of persistent inhibition because the effective duration of inhibition ended 32.39 ms before the offset of the 50-ms NE tone. The average baseline spike count, determined at the first 10 ISIs, was 0.70 ± 0.70 spikes/stimulus compared to the 0.24 ± 0.50 spikes/stimulus displayed between T_1 and T_2.

Comparing the BEF and non-BEF conditions, the change in frequency away from BEF weakened the inhibition evoked by the NE tone. In the case of Figure 6, inhibition was abolished when the NE tone was at a non-BEF. This suggests that the inhibition is stronger or longer in binaural paired-tone stimulation when the NE tone is at the BEF of the cell, compared to when the NE tone is at a non-BEF.

In the 38 DTNs that were tested, spike suppression was reliably observed in the monotic condition (38 of 38; 100%), however, only 20 cells (53%) displayed spike suppression during binaural paired-tone stimulation when both the BD and NE tone were at BEF. Moreover, only 9 cells (23%) showed evidence of inhibition at each of the 5 standardized NE frequencies which included frequencies both within and outside the 50% eBW ($0.5L_{eBW}$, $1.5L_{eBW}$, $0.5H_{eBW}$, $1.5H_{eBW}$).

Leading inhibition was exhibited in 20% (13/64) of the binaural paired-tone trials, compared to 63% (24/38) of trials in the monaural condition. There was no significant

difference in the proportion of cells among conditions with leading [$\chi^2(4) = 5.571$, $p = 0.234$, $n = 64$] and persistent [$\chi^2(4) = 2.595$, $p = 0.628$, $n = 64$] inhibition across the five standardized NE tone frequencies in the binaural condition. When the monaural BEF condition was included, there was a significant difference between the dichotic and monotic conditions the duration of both leading [$\chi^2(5) = 62.565$, $p << 0.01$, $n = 102$] and persistent [$\chi^2(5) = 84.209$, $p << 0.01$, $n = 102$] inhibition. These findings show that inhibition was very similar across the 5 tested NE tones that were within 150% of the eBW of the cell. Figure 8A shows that the latency of the inhibition was essentially constant across the dichotic condition, but was significantly different from the monotic condition. When cells were tested in the monotic condition with the BD and NE tones set to the BEF, most neurons displayed leading inhibition. But when the same cells were tested with dichotic stimulation, most showed evidence of lagging, or no inhibition (Fig. 8A).

Similarly, persistent inhibition (Fig. 8B) was generally observed in the monotic condition but was abolished in the dichotic condition, with the mean values of persistent inhibition remaining relatively constant across the 5 standardized NE tone frequencies with one exception. A change occurred at $1.5H_{eBW}$, with sustained inhibition moving away from the mean of the other NE frequency values. The duration of inhibition was shorter than the NE tone duration. In both panels of Figure 8, an inverted-U shape was somewhat apparent, especially in Fig. 8A. There was a significant difference between the monotic and dichotic conditions in the durations of both leading and persistent inhibition (Table 1).

Comparing leading/lagging inhibition to BD, FSL, and duration filter class

A correlation was displayed between leading inhibition and BD for the 5 standardized NE tone frequencies in the dichotic and monotic conditions (Figure 9A). Leading inhibition and BD were positively correlated in the monotic condition ($R = 0.496$, $p = 0.002$), but there was no correlation between the duration of leading inhibition and BD in any of the 5 dichotic

frequency conditions (Table 2). Leading inhibition was significantly larger in band-pass DTNs compared to short-pass DTNs in the monotic condition (band-pass = 5.95 ± 4.13 ms; short-pass = 0.01 ± 2.93 ms; Mann–Whitney U = 49.00, p << 0.01), but there was no correlation between leading inhibition and duration class in the dichotic condition except at the $1.5L_{eBW}$ (band-pass = 1.02 ± 2.98 ms; short-pass = -6.73 ± 5.40 ms; Mann–Whitney U = 81.00, p = 0.038) (Table 3).

There was a positive correlation between the amount of leading inhibition and FSL (Fig. 9B) in the monotic condition (R = 0.46, *p* = 0.004) (Table 2), but there was no correlation between leading inhibition and FSL in the 5 dichotic conditions, similar to previous studies (Sayegh *et al.*, 2014).

Differences in FRAs, BWs, and Q factors between excitatory and inhibitory best frequencies

By systematically varying the frequency of the BD tone we were able to construct an eFRA and measure the BEF and eBW of each cell. Binaural paired-tone stimulation at the 5 standardized NE tone frequencies allowed us to construct iFRA and measure the BIF and iBW for each cell. Of the 16 cells tested, 11 (68.8%) had iBWs that were narrower than the cell's eBW. Figure 10A is an example eFRA measured from a DTN with a BEF of 44.0 kHz and an eBW of 9 kHz (ranging from 38.0 to 47.0 kHz; or -0.21 to +0.095 octaves re BEF). The excitatory $Q_{10\,dB}$ value was 4.89. The normalized duration of inhibition, derived from dichotic paired-tone testing is shown in Figure 10B; the cell had a BIF of 44.0 kHz and an iBW of 4.2 kHz (relative to the eBW, ranging from 42.5 to 46.7 kHz; or -0.05 to +0.08 octaves re BEF). The iBW was smaller than the eBW. The inhibitory Q value was 10.47. The low- and high-frequency slopes of the normalized iFRA were $Slope_{Low}$ = 9.82 and $Slope_{High}$ = -5.08. In this cell, the normalized duration of inhibition demonstrated that the strength of inhibition increased more quickly at low *versus* high frequencies. Excitation in this DTN was

more broadly tuned than neural inhibition as the cell's iBW was narrower and was completely overlapped by the eBW (compare width of grey boxes in Fig. 10A and 10B).

Examples of a DTN where the BEF did not match the BIF are shown in Figure 10C and 10D. Figure 10C displays an eFRA determined from a DTN with a BEF of 54.0 kHz and an eBW of 12 kHz (ranging from 49.0 to 61.0 kHz; or -0.14 to 0.18 octaves re BEF). The excitatory $Q_{10\ dB}$ value was 4.5. The same cell in Figure 10D had a BIF of 51.5 kHz and a iBW of 3.5 kHz (ranging from 49.2 to 52.7 kHz; or -0.07 to 0.03 octaves re BIF). The inhibitory Q value was 14.64. The low- and high-frequency slopes of the normalized iFRA were $Slope_{Low}$ = 6.72 and $Slope_{High}$ = -14.72. In this DTN, the normalized duration of inhibition decreased more quickly at high *versus* low frequencies. Similar to the previous example, neural excitation was more broadly tuned than neural inhibition as the cell's iBW was narrower and was completely overlapped by the cell's eBW (compare grey boxes in Fig. 10C and 10D). These data provide evidence that the strength of inhibition was maintained only over a narrow range of NE tone frequencies.

We compared the difference between the BIF and BEF got each cell and the mean difference was 0 octaves (median difference = 0.00 octaves, mean = 0.00 ± 0.05 $p > 0.05$, n = 16). This means the monaural BEF matched the BIF for the ipsilateral ear in DTNs. There were 7 cells (43.75%) in which the BIF and BEF exactly matched, 3 cells (18.75%) that differed by only ±0.05 octaves from BEF, and 6 cells (37.5%) were found to be ±0.12 octaves from BEF (Fig. 11). This shows that there was a close correspondence in the sound frequencies evoking maximum synaptic excitation and inhibition in DTNs.

We compared the sharpness of excitatory and inhibitory tuning between the contralateral and ipsilateral inputs to a DTN using excitatory $Q_{10\ dB}$ and inhibitory Q factors measured from the same DTNs. There was a significant difference in the distribution of the average excitatory (10.11 ± 9.99) and inhibitory (14.89 ± 8.31) Q factors across the population of DTNs tested (Fig. 12; Z = -2.327, p = 0.02, n = 16). There were 5 cells

(31.25%) that had an excitatory $Q_{10\ dB}$ factor that was larger than the corresponding inhibitory Q factor. The remaining 11 cells (68.75%) had inhibitory Q factors that were larger than the corresponding excitatory $Q_{10\ dB}$. These findings demonstrate that ipsilateral inhibitory inputs to DTNs were more sharply tuned than the contralateral excitatory inputs. Finally, we compared the low-frequency slope ($Slope_{Low}$) to the absolute value of the high-frequency slope ($|Slope_{High}|$) of the iFRA to examine the symmetry of the ipsilaterally-evoked inhibitory tuning re BEF. There was no difference between the $Slope_{Low}$ values (mean = 11.62 ± 7.18) and the $|Slope_{High}|$ values (mean = 17.46 ± 17.18) across the population DTNs tested (Z = -1.293, p = 0.196, n = 16). There were 6 cells (37.5%) that had a $Slope_{Low}$ that was steeper than $|Slope_{High}|$. The remaining 10 cells (62.5%) had $|Slope_{High}|$ steeper than the corresponding $Slope_{Low}$ (Fig. 13). These data indicate that there were no differences in the strength of ipsilaterally-evoked inhibition at sound frequencies below and above the BIF.

Discussion

Monaural and binaural inhibitory inputs to DTNs

Electrophysiological studies have allowed researchers to develop neural circuit models that explain the origin of DTNs, with the primary finding that there is a temporal interaction between excitatory and inhibitory synaptic inputs (Casseday *et al.,* 1994, 2000; Covey *et al.,* 1996; Faure *et al.,* 2003; Fuzessery & Hall, 1999; Aubie *et al.,* 2009; Sayegh *et al.,* 2012; Valdizón-Rodríguez & Faure, 2017). There are situations in which a duration-selective circuit does not require inhibition to be affecting the cell. If an onset- and offset-evoked subthreshold excitatory input occur at the same time then the membrane potential of a DTN can become suprathreshold (Aubie *et al.,* 2009; Sayegh *et al.,* 2011). Application of pharmacological antagonists of inhibitory neurotransmitters has been shown to manipulate s cell's duration selectivity, either broadening or abolishing it (Casseday *et al.,* 1994, 2000; Jen & Feng, 1999; Jen & Wu, 2005; Yin *et al*., 2008). There are also findings from extracellular

single-unit recordings and whole-cell patch-clamp recordings that have identified a monaurally onset-evoked inhibitory input in DTNs that occurs prior to an onset-evoked excitatory input in DTNs. Studies also found that DTNs receive a sustained inhibitory input that lasts as long, or longer than the duration of the stimulus that evoked the inhibition (Casseday *et al.*, 1994, 2000; Covey *et al.*, 1996; Ehrlich *et al*., 1997; Faure *et al.*, 2003). Altogether, the previous studies have provided sufficient evidence that the formation of duration selectivity is caused by neural inhibition influencing excitatory responses.

The present study used single-unit recording paired-tone stimulation, combined with methods used by Faure *et al.* (2003), to measure the latency, strength, and duration of the monaurally-evoked inhibition acting on DTNs. The paradigm involves a roving BD tone that consistently elicits spiking responses and a NE tone that is not excitatory. The NE tone suppresses spiking that would otherwise be evoked by the roving BD tone. It is important to emphasize that both the BD and NE tones activate both excitatory and inhibitory synaptic inputs (Casseday *et al.*, 1994, 2000; Fuzessery & Hall, 1999). Faure *et al.* (2003) described the net effect of both tones with the NE tone being inhibitory and the BD tone being excitatory. This method works because in addition to being non-excitatory, the NE tone recruits inhibition that suppresses spiking evoked by the roving BD tone.

We tested DTNs, of which 100% displayed spike suppression and/or deviations in spike latency during monotic paired-tone stimulation (i.e. when the tone pair as presented to the contralateral ear). In the dichotic conditions 52.6% displayed inhibition when the NE tone was presented to the ipsilateral ear and the BD tone was presented to the contralateral ear. In both conditions, the tones were matched in amplitude (e.g. Figures 6 and 7). These results are consistent with a previous binaural paired-tone stimulation study conducted by Sayegh *et al*. (2014), who found that about 50% of DTNs did not exhibit spike suppression when the NE tone stimulated the ipsilateral ear. Moreover, when inhibition was observed it typically occurred after the FSL in the dichotic condition, showing that the inhibition evoked

ipsilaterally lagged behind the excitation evoked contralaterally (Figure 8A, Table 1). These findings further supporting the theory that the neural circuits involved with the creation of the temporally-selective responses of DTNs are monaural (Faure *et al.*, 2003; Sayegh *et al.*, 2014).

Persistent inhibition occurred in all cases of monaural paired-tone stimulation. In the dichotic condition, the duration of inhibition was typically significantly shorter than the duration of the NE tone (Figure 8B, Table 1). This information regarding persistent inhibition remains consistent with previous findings (Faure *et al.*, 2003; Sayegh *et al.*, 2014). These findings further support the idea that monaural circuits provide the inhibition needed to create duration selectivity in the mammalian auditory midbrain.

Altogether, it is clear that the ipsilateral inhibition in DTNs is weaker, slower, and is shorter in duration compared to the inhibition that is evoked contralaterally. This suggests that contralateral (monaural) pathways create duration selectivity and ipsilateral (binaural) pathways shape the responses (Figure 8, Table 1). Because ipsilateral inhibitory inputs to DTNs seem to be independent of contralateral inputs, this further supports the notion that DTNs are primarily created by monaural circuits.

Varying the NE tone frequency in monotic and dichotic paired-tone stimulation

There were some differences in the time course of the evoked inhibition in DTNs during binaural paired-tone stimulation across the 5 standardized NE tone frequencies (Figure 2). Valdizón-Rodríguez & Faure (2017) manipulated the NE tone frequency during monaural paired-tone stimulation and found that there were no differences in the onset of inhibition. Additionally, there were no correlations between leading inhibition and BD, FSL and duration filter class between the 5 standardized NE tone frequencies. They also found that varying the NE tone frequency changed the sustained inhibition that was evoked, with the duration decreasing systematically as the NE tone frequency moved away from the BEF. Our findings

indicate that inhibition occurred in fewer cells as the NE tone frequency moved away from BEF. The BEF condition had inhibition in >50% of the cells, while inhibition occurred at all 5 frequency points in only <25% of cells. This is important to note, as differences in leading and persistent inhibition between NE tone frequencies could have been more apparent if more cells had inhibition at each of the 5 standardized frequencies. Regardless, our research found that there was no difference in the durations of leading and persistent inhibition between the 5 standardized NE tone frequencies (Fig. 8). With additional cells, we hypothesize that the BEF could yield the shortest value of lagging inhibition, while the points outside the eBW would exhibit longer delays before inhibition occurred.

Leading inhibition has been suggested to be a general property of central auditory processing because of the role it has in temporal processing, binaural hearing, and encoding frequency modulated sweeps (Galazyuk *et al*., 2005; Carney and Yin, 1989; Razak and Fuzessery, 2006). Previously, computational studies and electrophysiological recordings have shown that the BD and duration filter class of a DTN depend on the amount of leading inhibition (Aubie *et al*., 2009; Ehrlich *et al.,* 1997). Sayegh *et al.* (2014) found differences in the correlation between leading inhibition and BD in monotic and dichotic conditions. The duration of leading inhibition led excitation in the monotic condition increased in DTNs tuned to longer BDs and FSLs, but this relationship was not observed in the dichotic condition. Valdizón-Rodríguez & Faure (2017) found that the correlation between leading inhibition and BD (or FSL and/or duration filter class) did not change across the 5 standardized NE tone frequencies in the monotic condition. They also found that the duration of leading inhibition increased in DTNs tuned to longer BDs and FSLs. This relationship occurred across all standardized frequencies, with the exception being that no relationship was found between leading inhibition and FSL at the highest NE tone frequency ($1.5H_{eBW}$). We found that leading inhibition was not related to the BD, FSL and duration filter class when evoked from the ipsilateral ear (Fig. 9, Table 2). These results expand on previous findings by identifying that

variations of the NE tone frequency in binaural recordings do not alter the characteristics of inhibition acting on DTNs. If binaural inputs act as a separate circuit that does not determine the cell's duration selectivity, it makes sense that the correlation between leading inhibition and BD (or FSL and/or duration filter class) would also be maintained across a cell's eFRA. An odd finding in our data was the significant difference between leading inhibition and duration filter class that was only apparent in the dichotic condition at $1.5L_{eBW}$. Once again, this difference may not exist if a larger number of cells had been tested. Together, these findings help to strengthen the hypothesis that BD, FSL and duration filter characteristics of DTNs are shaped from monaural inhibition that is evoked by stimulation of the contralateral ear.

Comparing the excitatory and inhibitory bandwidths

We measured a cell's iFRA and iBW during the dichotic paired-tone stimulation, and fount that the majority of DTNs had ipsilaterally-evoked inhibition that was more narrowly tuned in frequency than contralaterally-evoked excitation, with a cell's eBW typically overlapping its iBW (Fig. 10, 11). Previous research has demonstrated that inhibition is required for duration selectivity in the IC (Casseday *et al*., 1994; Fuzessery and Hall, 1999; Jen and Wu, 2005; Yin *et al.*, 2008). Additionally, Valdizón-Rodríguez & Faure (2017) identified that the iBWs of DTNs were broader than the corresponding eBWs when stimulating the contralateral ear. This difference in frequency selectivity was hypothesized to preserve temporal selectivity across the eFRA. A DTN should receive an inhibitory input whose spectral tuning is as broad as or broader than the tuning of its excitatory input. This could help to preserve duration tuning at non-BEFs. Our finding that the ipsilateral iBW was more sharply tuned than contralateral eBW (re IC being recorded), has been reported in a previous study recording from non-DTNs in the IC of *E. fuscus* (Lu and Jen, 2003). This narrower tuning (re to the monotic condition) shows that temporal selectivity cannot be

maintained across a wide range in the dichotic condition because the cell does not receive inhibition throughout its eFRA. The slopes flanking the iBW did not differ significantly (Fig. 13). This finding is interesting as it displays a clear difference between slope measurements in binaural and monaural paired-tone stimulation and monaural temporal masking studies. In monaural studies the slopes flanking the iBW would differ, as the high-frequency slope would be much steeper than the low-frequency slope (Valdizón-Rodríguez & Faure, 2017; Bacon & Moore, 1986). In a binaural study conducted by Brosch & Schreiner (1997), auditory neurons were presented with stimuli of pure tones of varying amplitudes. The slopes corresponding to latency of the probe responses tended to change with stimulus level. Many cells would exhibit long periods of inhibition after the masker at an intermediate intensity, and there did not tend to be differences in slopes on the flanks of the response area. Slopes of latency responses to the probe were calculated differently in our study. We found the high-frequency slope of iFRA did not differ from the low-frequency slope, unlike monaural studies. It is unclear whether this finding would change if a larger number of cells were tested.

Role of ipsilateral inhibition

Similar to previous studies, our results showed that the inhibition evoked by ipsilateral stimulation differed from the inhibition evoked by contralateral stimulation (Lu & Jen, 2003; Sayegh *et al.*, 2014). When stimulating the ipsilateral ear, a subset of DTNs received inhibition that was weaker and shorter lasting relative to the inhibition evoked from stimulating the contralateral ear (Fig. 5). Varying the frequency of the NE tone in the ipsilateral ear resulted in large changes in the latency and duration of the evoked inhibition compared to contralateral (monaural) inhibition. We also found some evidence that the strength of inhibition was weaker when the NE tone frequency was further away from the cell's BEF. The fact that DTNs can receive, in some cases, two different inhibitory inputs, one ipsilateral which is weaker and that interacts with the other inhibition that is evoked

monaurally (contralateral) is interesting. Neuropharmacological studies (Casseday *et al.,* 2000; Lu & Jen 2001; Yin *et al.,* 2008) have identified at least two neurotransmitters that may be responsible for the inhibition acting on midbrain DTNs: γ-aminobutyric acid (GABA) and glycine. In those studies, application of one neurotransmitter blocker (e.g. GABA or glycine) typically broadened excitatory frequency tuning curves and decreased duration selectivity. When both antagonists (e.g. GABA and glycine) were administered, inhibition was blocked and duration selectivity was abolished. It's possible that the different inhibitory inputs to DTNs (i.e. contra- and ipsilateral) use different inhibitory neurotransmitters. The ventral nucleus of the lateral lemniscus (VNLL) and the dorsal nucleus of the lateral lemniscus (DNLL) have both been identified as structures that provide neural inhibition to the IC, contralaterally and ipsilaterally respectively (Covey & Casseday 1986; Li & Kelly, 1992; Schneiderman *et al.,* 1993). Additionally, VNLL neurons are primarily monaural, whereas DNLL neurons are primarily binaural (Covey & Casseday, 1991; Li and Kelly, 1992). Finally, it seems that VNLL neurons utilize GABAergic and glycinergic inputs (Casseday *et al.,* 1994), while the DNLL only has GABAergic inputs (Schneiderman *et al.,* 1993). This helps to explain the dual neurotransmitter inputs to the IC and why the use of both antagonists resulted in the blocking of all inhibition.

Our study found distinct difference in leading and persistent inhibition between monotic and dichotic paired-tone testing. It is known that VNLL neurons participate in spectro-temporal processing. Shown in our results all DTNs tested with monaural paired-tone stimulation exhibited inhibition. It is possible that the inhibition evoked in DTNs from the ipsilateral ear is directly linked to DNLL neurons and that these DTNs do not play a significant role in temporal tuning. With ~50% of DTNs showing inhibition during binaural paired-tone stimulation, it is likely that these inputs from the DNLL are not the primary determinant of duration selectivity assuming that DTNs receive input from the DNLL. Our results show that DTNs can receive at least two types of inhibitory inputs. The first type is

monaural in nature, is observable in all cells, and is evoked by stimulation of the contralateral ear. This inhibition is responsible for creating the temporally selective responses characteristic of DTNs. The second type of inhibition is only seen in a subset of DTNs and is evoked from stimulation of the ipsilateral ear. Future studies may wish to test the hypothesis that the two inhibitory inputs to DTNs may use different neurotransmitters (GABA or glycine).

Monotic and dichotic temporal masking

The present study used monotic and dichotic paired-tone stimulation to characterize the properties of neural inhibition acting on midbrain DTNs in bats. The acoustic stimulation technique mimics the design utilized in auditory psychophysical experiments that study temporal masking. The excitatory BD tone is analogous to a probe signal, while the NE tone is analogous to a masker signal. Neural correlates of auditory temporal masking pattern have been identified in DTNs with paired-tone stimulation (Faure *et al.*, 2003; Sayegh *et al.*, 2014; Valdizón-Rodríguez & Faure, 2017). The change in spike count and latency that occurred when the BD tone preceded the NE tone is the neural equivalent of backward masking and was caused by the leading inhibition evoked by the NE tone that suppressed the spikes evoked by the BD tone. Similarly, spike suppression when the BD and NE tones overlapped in time to form a single composite signal are the neural equivalent of simultaneous masking and was caused by the sustained inhibition evoked by the NE tone that suppressing the spikes evoked by the BD tone. Finally, spike suppression that occurred when the BD tone followed the NE tone is the neural equivalent of forward masking and occurred as a result of the persistent inhibition evoked by the NE tone altering spikes evoked by the BD tone.

We found that the ipsilaterally-evoked lagging and sustained inhibition as weaker and shorter in duration than the leading and persistent inhibition evoked contralaterally (Fig. 5). Additionally, the ipsilateral inhibition was more narrowly tuned compared to the contralaterally evoked inhibition (Fig. 8). Finally, the frequency tuning of the ipsilateral

inhibition was narrower than that of its contralateral excitatory counterpart (Fig. 10). Psychophysical masking trials have shown that the duration of forward and backward masking were longer during monotic stimulation compared to dichotic stimulation (Elliot, 1962a,b; Deatherage & Evans, 1969). One paper provided evidence that contralateral masking tuning curves were sharper than their ipsilateral counterpart (Mills *et al.*, 1996), a finding that is contrary to the results of the present neural study on the physiology of DTNs.

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Figures

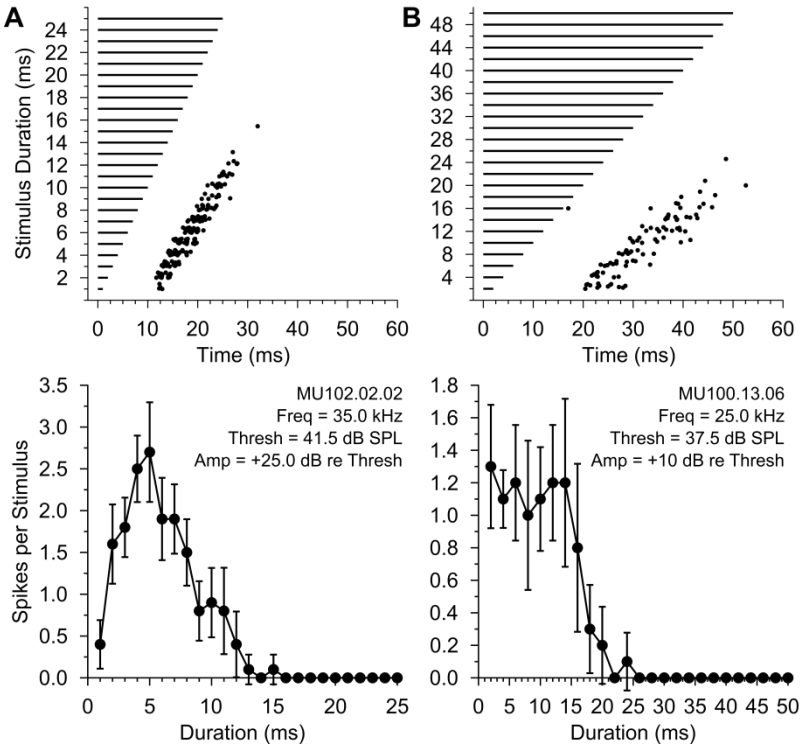

Fig. 1: Two examples of duration-tuned neurons (DTNs) recorded from the inferior colliculus (IC) of the big brown bat. *A*: Band-pass DTN with a best duration of 5 ms. *B*: short-pass DTN with a best duration of 1 ms. *Top*: dot raster displaying the timing of evoked action potentials in response to suprathreshold best excitatory frequency (BEF) tones that were randomly varied in duration. *Bottom*: Mean ± standard error (SE) spikes per stimulus as a function of stimulus duration for suprathreshold BEF tones. Stimulus levels were +25 dB (A) and +10 dB (B) re threshold. n = 10 trials per stimulus.

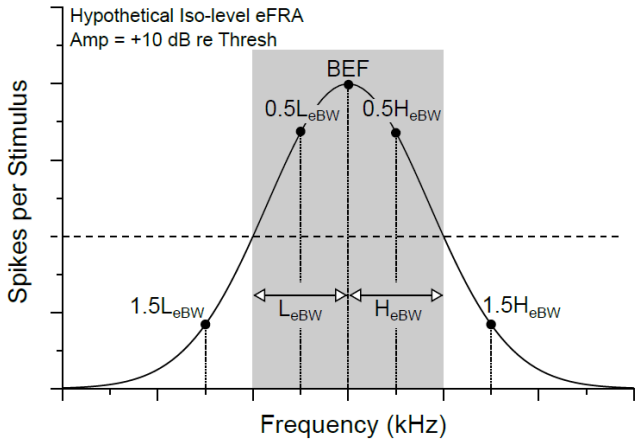

Fig. 2: Determining the 5 standardized NE tone frequencies. Duration-selective neurons were tested with paired-tone stimulation using 5 NE tone frequencies that were standardized relative to the eBW (gray box) of each cell. The figure illustrates a hypothetical isolevel eFRA of a DTN, obtained by recording the number of spikes evoked in response to single BD tones that were varied in frequency and presented at +10 dB above threshold. The cutoffs of the eBW (edges of gray box) were defined as the lowest and highest frequencies that evoked 50% (horizontal dashed line) of the maximum spike count at the BEF. Linear interpolation as used to determine 50% cuttoffs. The eBW was divided into lower (L_{eBW}) and higher (H_{eBW}) spectral partitions re the BEF (white arrows), and the 5 standardized NE tone frequencies (black dots) were selected as (1) 1.5 times the L_{eBW} below the BEF ($1.5L_{eBW}$), (2) the midpoint of the L_{eBW} ($0.5L_{eBW}$), (3) the BEF, (4) the midpoint of the H_{eBW} ($0.5H_{eBW}$), and (5) 1.5 times the H_{eBW} above the BEF ($1.5H_{eBW}$). Therefore, each cell was tested with 3 NE tone frequencies within its 50% eBW (BEF, $0.5L_{eBW}$, $0.5H_{eBW}$) and 2 NE tone frequencies outside its 50% eBW ($1.5L_{eBW}$, $1.5H_{eBW}$). Additional NE tone frequencies were tested whenever possible (not shown). Reprinted from Valdizon-Rodriguez & Faure, 2017.

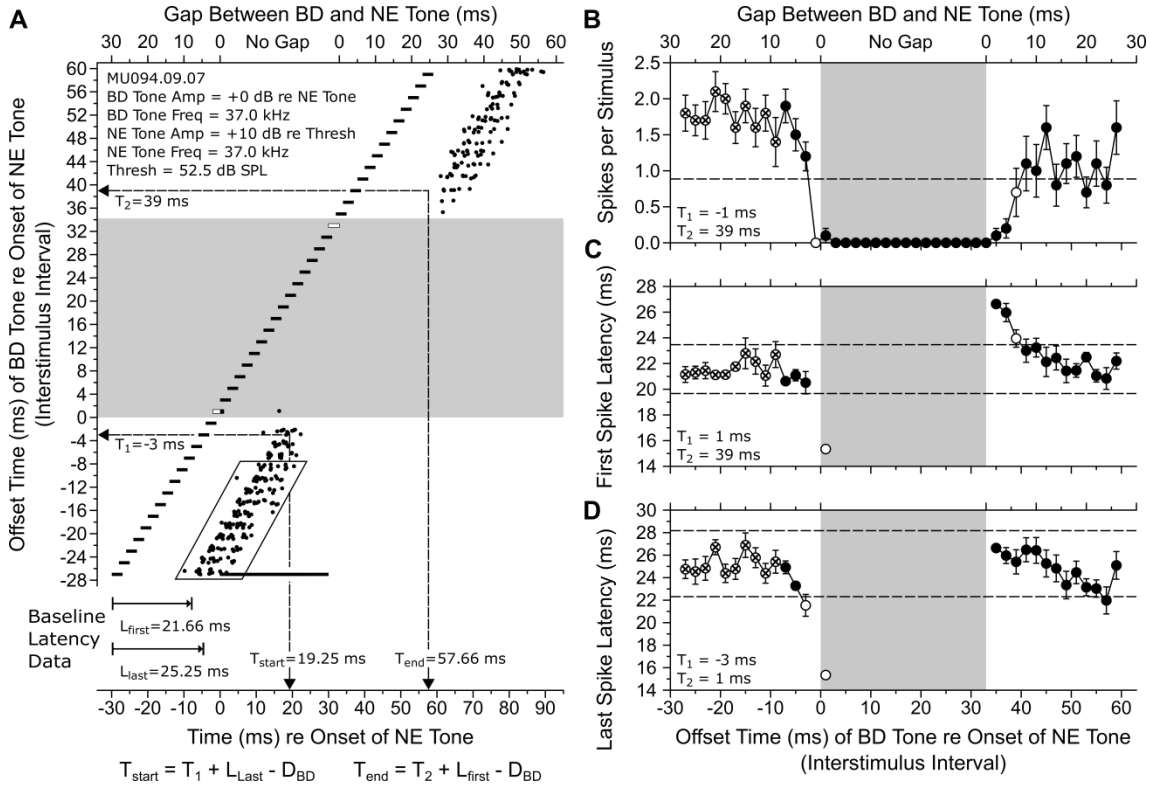
A
Gap Between BD and NE Tone (ms)
No Gap
Offset Time (ms) of BD Tone re Onset of NE Tone (Interstimulus Interval)
MU094.09.07
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 37.0 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 37.0 kHz
Thresh = 52.5 dB SPL
T2=39 ms
T1=-3 ms
Baseline Latency Data
Lfirst=21.66 ms
Llast=25.25 ms
Tstart=19.25 ms
Tend=57.66 ms
Time (ms) re Onset of NE Tone
Tstart = T1 + LLast - DBD
Tend = T2 + Lfirst - DBD
B
Spikes per Stimulus
T1 = -1 ms
T2 = 39 ms
C
First Spike Latency (ms)
T1 = 1 ms
T2 = 39 ms
D
Last Spike Latency (ms)
T1 = -3 ms
T2 = 1 ms
Offset Time (ms) of BD Tone re Onset of NE Tone (Interstimulus Interval)

Fig 3: Measuring the time course of inhibition with paired-tone stimulation. *A*: Dot raster display showing how changes in a cell's spike count and/or latencies evoked by the roving BD tone were used to infer the time course of the synaptic inhibition evoked by the NE tone using the equations displayed at *bottom*. The BD tone (3-ms *black bars*) onset time was randomly varied relative to the onset time of the NE tone (30-ms *black bar*; drawn once for clarity). *Bottom x-axis* displays time relative to the onset of the NE tone; *top x-axis* displays the ISI or gap between the BD and NE tones. The *y-axis* shows the offset time of the BD tone relative to the onset time of the NE tone. In monotic recordings, the 2 tones were electronically mixed and presented monaurally to the ear contralateral to the IC recorded. In dichotic recordings, the BD tone was presented to the contralateral ear while the NE tone was presented to the ipsilateral ear. When the 2 tones were temporally contiguous or overlapping (*gray box*), a single compound stimulus with an amplitude pedestal resulted. A BD tone bar with a white fill indicates when the BD and NE tones were contiguous. Responses from the first 10 trials with the longest ISIs (spikes in parallelogram) were used to calculate the mean ± SD baseline spike count (1.76 ± 0.80 spikes/stimulus), FSL (L_{first} = 21.66 ± 2.06 ms), and LSL (25.25 ± 2.93 ms); data points averaged in the baseline calculation are shown as *white circles with an X*. During paired-tone stimulation BD tone-evoked responses were suppressed by inhibition evoked by the NE tone. Three criteria were used to determine the effective onset (T_1) and offset (T_2) times of the NE tone evoked inhibition based on a 50% change in the cell's spike count and/or a 1 SD change in the FSL and/or LSL (see Methods). The first ISI with a significant deviation from the baseline spike count and/or latency was T_1 = -3 ms and was measured with a LSL criterion. The last ISI with a significant deviation from the baseline spike count or latency was T_2 = 39 ms and was measured with either a spike count and/or FSL criterion. With the equations shown, T_{start} = 19.25 ms and T_{end} = 57.66 ms, resulting in an effective duration of inhibition of 38.41 ms. *B*: Mean ± SE spikes per stimulus plotted as a function of the ISI between the BD and NE tones. *Dashed line* represents 50% of the baseline

spike count. Leftmost *open circle* is the first ISI with an evoked spike count ≤50% of the baseline spike count (T_1 = -1 ms). Rightmost *open circle* shows the last ISI with an evoked spike count ≤50% of baseline (T_2 = 39 ms). *C*: Mean ± SE FSL as a function of the ISI between the BD and NE tones. *Dashed lines* represent ±1 SD from the baseline FSL. Leftmost *open circle* shows the first ISI when the FSL deviated by >1 SD from baseline (T_1 = 1 ms). Rightmost *open circle* shows the last ISI when the FSL remained deviated by >1 SD from baseline (T_2 = 39 ms). *D*: Mean ± SE LSL as a function of the ISI between the BD and NE tones. Dashed lines represent ±1 SD from the baseline LSL. Leftmost *open circle* shows the first ISI when the LSL deviated by >1 SD from baseline (T_1 = -3 ms). Rightmost *open circle* shows the last ISI when the LSL remained deviated by >1 SD from baseline (T_2 = 1 ms). The final values of T_1 (-3 ms) and T_2 (39 ms) were those that most sensitively reflected the time course of the NE tone-evoked inhibition. n = 10 trials per stimulus. Reprinted from Valdizon-Rodriguez & Faure (2017).

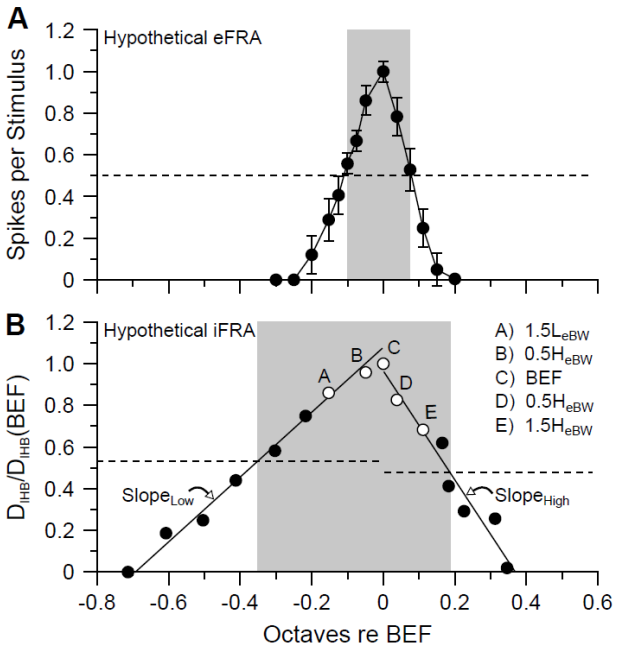
A
Hypothetical eFRA
Spikes per Stimulus
1.2
1.0
0.8
0.6
0.4
0.2
0
B
Hypothetical iFRA
A) 1.5L$_{eBW}$
B) 0.5H$_{eBW}$
C) BEF
D) 0.5H$_{eBW}$
E) 1.5H$_{eBW}$
A
B
C
D
E
Slope$_{Low}$
Slope$_{High}$
D$_{IHB}$/D$_{IHB}$(BEF)
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
Octaves re BEF

Fig 4: Determining the excitatory and inhibitory FRAs, BEF, eBW, BIF, and iBW. *A*: Hypothetical isolevel eFRA of a DTN showing the mean ± SD spikes per stimulus in response to single BD tones randomly varied in frequency and presented at +10 dB re threshold (*filled circles*). The eBW (*gray box*) was defined as the span between the lowest and highest frequencies at the 50% spike count re BEF (*horizontal dashed line*). See Fig. 2 for additional details. *B*: Hypothetical isolevel iFRA of the same cell, measured as the duration of inhibition (D_{IHB}) evoked at each NE tone frequency at +10 dB re threshold (relative to the BEF) normalized by the duration of inhibition evoked at the BEF at +10 dB re threshold [i.e., $D_{IHB}/D_{IHB}(BEF)$]. All cells were tested with at least 5 standardized NE tone frequencies described in Fig. 2 (*open circles A–E*), and whenever possible additional NE tone frequencies were presented (*filled circles*). Except for the BEF, the number of NE tone frequencies tested was independent of the number of BD tone frequencies in the eFRA. The BIF was defined as the frequency evoking the largest normalized duration of inhibition (*open circle*) *C*). Separate linear regressions (*solid lines*) were computed for the low ($Slope_{Low}$)- and high ($Slope_{High}$)-frequency tuning slopes of the iFRA. Each regression was interpolated to 50% of its maximum normalized duration of inhibition at the BIF, and this could result in a slightly different 50% criterion (*horizontal dashed lines*) to define the lowest and highest cutoff frequencies (edges of *gray box*). For the hypothetical cell illustrated, the eBW ranged from -0.10 to 0.08 octaves (re BEF), and the iBW ranged from -0.35 to 0.19 octaves (re BEF). Cutoff frequencies were interpolated. Reprinted from Valdizon-Rodriguez & Faure (2017).

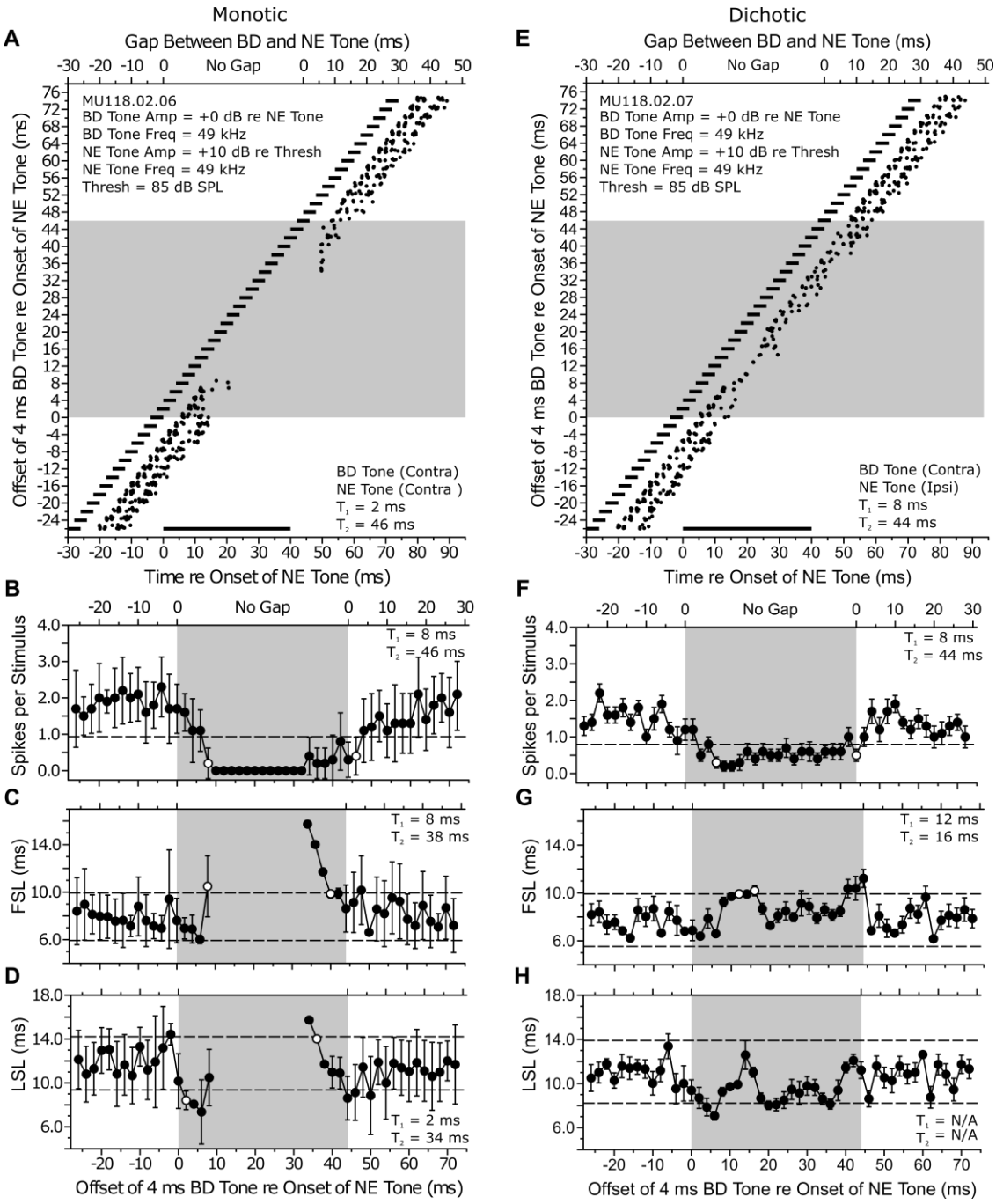
Monotic
Dichotic
A
E
Gap Between BD and NE Tone (ms)
No Gap
MU118.02.06
MU118.02.07
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 49 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 49 kHz
Thresh = 85 dB SPL
Offset of 4 ms BD Tone re Onset of NE Tone (ms)
BD Tone (Contra)
NE Tone (Contra)
NE Tone (Ipsi)
T1 = 2 ms
T2 = 46 ms
T1 = 8 ms
T2 = 44 ms
Time re Onset of NE Tone (ms)
B
F
Spikes per Stimulus
T1 = 8 ms
T2 = 46 ms
T2 = 44 ms
C
G
FSL (ms)
T1 = 8 ms
T2 = 38 ms
T1 = 12 ms
T2 = 16 ms
D
H
LSL (ms)
T1 = 2 ms
T2 = 34 ms
T1 = N/A
T2 = N/A
Offset of 4 ms BD Tone re Onset of NE Tone (ms)

Fig. 5: Comparing monotic and dichotic paired tone stimulation. Significant change was said to occur when the evoked spike count/latency at two consecutive ISIs was <50% or 1 SD from the baseline response. Dot raster display illustrating spike suppression in a DTN in response to monotic (*left column*) and dichotic (*right column*) paired tone stimulation. *A*: When both tones were presented to the contralateral ear, spike suppression occurred when the 4 ms BD tone and the 40 ms NE tone were close in time. *B:* Mean ± SE spikes per stimulus as a function of the ISI between the BD and NE tones. The first ISI in which the spike count was ≤50% of baseline was T_1 = 8 ms. The longest ISI, starting from T_1, in which the spike count remained ≤50% of baseline was T_2 = 46 ms. *C:* Mean ± SE FSL as a function of the ISI between the BD and NE tones. The shortest ISI in which the FSL deviated by >1 SD from baseline was T_1 = 8 ms, and the longest ISI in which the FSL deviated by >1 SD from baseline was T_2 = 38 ms. *D:* Mean ± SE LSL as a function of the ISI between the BD and NE tones. The shortest and longest ISIs in which the LSL deviated by >1 SD from baseline was T_1 = 2 ms and T_2 = 34 ms, respectively. In the monotic condition, the earliest T_1 time was 2 ms and the longest T_2 time was 46 ms there were determined using a change in LSL and spike count, respectively. In the monotic condition, the latency of inhibition lagged the excitatory FSL by 1.78 ms. The inhibition persisted 0.22 ms longer than the NE tone and the duration of inhibition was 40.22 ms. *E:* Dot raster display illustrating responses of the same DTN to dichotic paired tone stimulation (BD tone to contralateral ear; NE tone to ipsilateral ear). The inhibition evoked by the NE tone caused a deviation in (*F*) the spike count, (*G*) FSL, but not in the (*H*) LSL of the cell. In the dichotic condition, the latency of inhibition lagged the excitatory FSL by 7.40 ms, and the duration of the inhibition was 32.60 ms. This example shows that the inhibition evoked by the NE tone in the dichotic condition was weaker, shorter in duration, and occurred at a longer latency than the inhibition evoked in the monotic condition. n = 10 trials per stimulus.

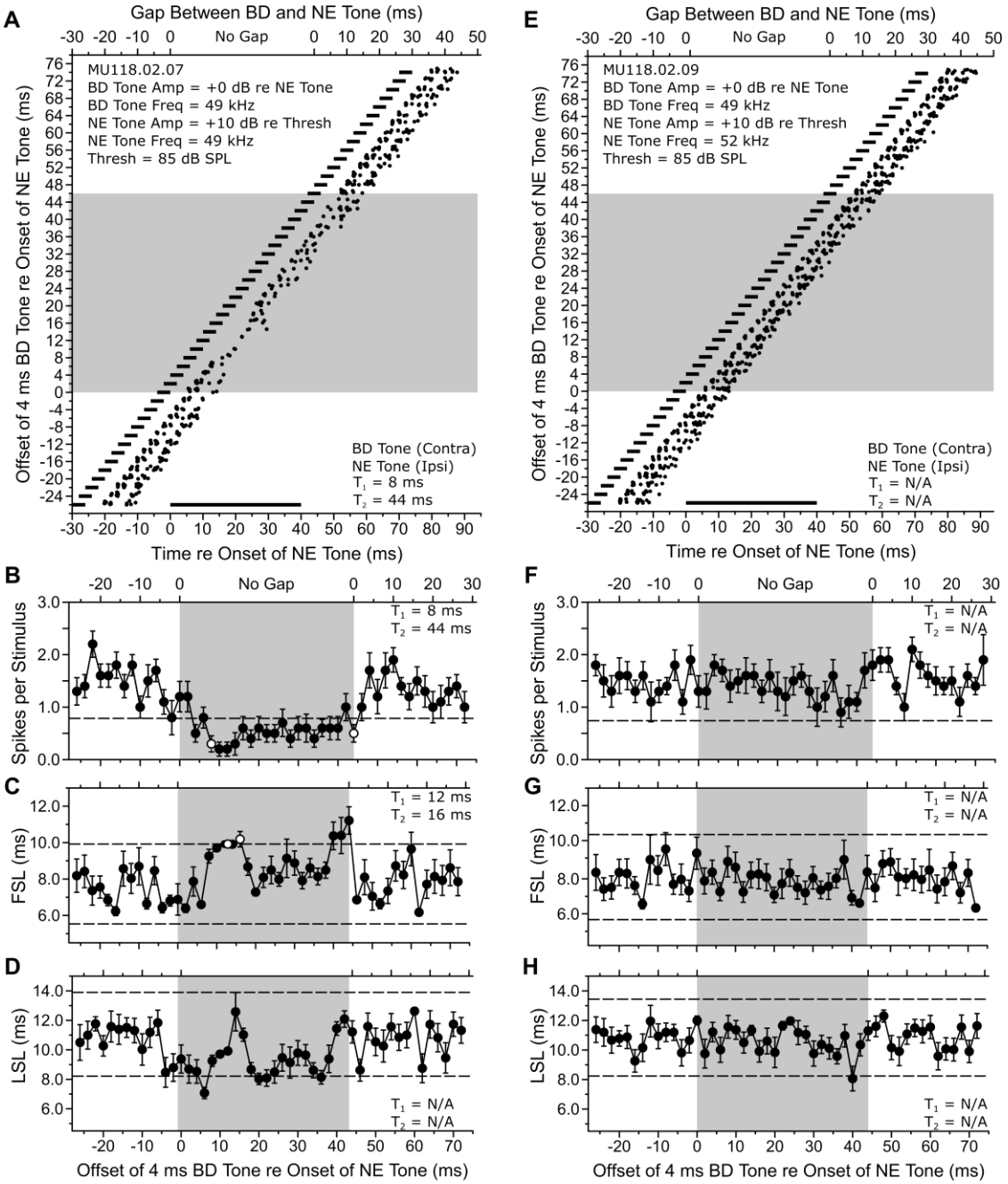
A
Gap Between BD and NE Tone (ms)
MU118.02.07
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 49 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 49 kHz
Thresh = 85 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = 8 ms
T2 = 44 ms
Offset of 4 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
B
Spikes per Stimulus
T1 = 8 ms
T2 = 44 ms
C
FSL (ms)
T1 = 12 ms
T2 = 16 ms
D
LSL (ms)
T1 = N/A
T2 = N/A
Offset of 4 ms BD Tone re Onset of NE Tone (ms)
E
Gap Between BD and NE Tone (ms)
MU118.02.09
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 49 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 52 kHz
Thresh = 85 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = N/A
T2 = N/A
Offset of 4 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
F
Spikes per Stimulus
T1 = N/A
T2 = N/A
G
FSL (ms)
T1 = N/A
T2 = N/A
H
LSL (ms)
T1 = N/A
T2 = N/A
Offset of 4 ms BD Tone re Onset of NE Tone (ms)

Fig. 6: Comparing NE tone evoked inhibition at the BEF (49 kHz) (*left column*) and at a non-BEF (52 kHz) (*right column*) during dichotic paired tone stimulation. Significant change was said to occur when the evoked spike count/latency at two consecutive ISIs was <50% or 1 SD from the baseline response. Dot raster display illustrating spike suppression in a short-pass DTN in response to the BEF condition *w*hen the BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear. *A:* Dot raster display illustrating responses of the same exact DTN shown in Figure 5 (*right side*). Dichotic paired tone stimulation was used (BD tone to contralateral ear; NE tone to ipsilateral ear). The inhibition evoked by the NE tone caused a deviation in (*B*) the spike count, (*C*) FSL, but not in the (*D*) LSL of the cell. *E*: Dot raster display depicting the responses of the same DTN when the NE tone was set to a non-BEF. There were no significant changes in the (*F*) spike count, (G) FSL, (*H*) or LSL. This example demonstrates that ipsilaterally evoked inhibition at the BEF was abolished when the NE tone was presented at a non-BEF that differed from the BEF by only 3 kHz or 0.086 octaves (re BEF). n = 10 trials per stimulus.

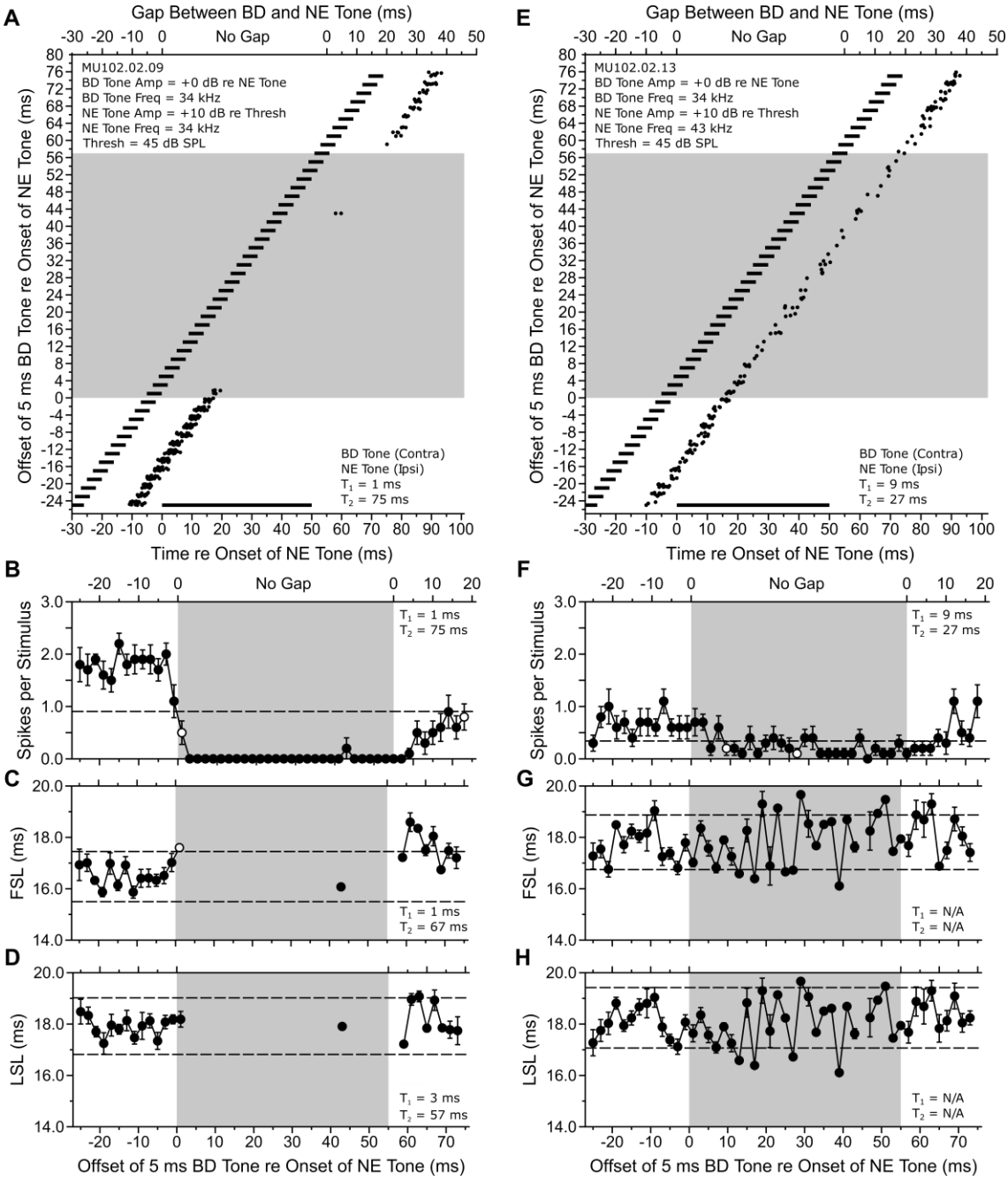
A
Gap Between BD and NE Tone (ms)
No Gap
MU102.02.09
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 34 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 34 kHz
Thresh = 45 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = 1 ms
T2 = 75 ms
Offset of 5 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
B
Spikes per Stimulus
T1 = 1 ms
T2 = 75 ms
C
FSL (ms)
T1 = 1 ms
T2 = 67 ms
D
LSL (ms)
T1 = 3 ms
T2 = 57 ms
Offset of 5 ms BD Tone re Onset of NE Tone (ms)
E
Gap Between BD and NE Tone (ms)
No Gap
MU102.02.13
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 34 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 43 kHz
Thresh = 45 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = 9 ms
T2 = 27 ms
Offset of 5 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
F
Spikes per Stimulus
T1 = 9 ms
T2 = 27 ms
G
FSL (ms)
T1 = N/A
T2 = N/A
H
LSL (ms)
T1 = N/A
T2 = N/A
Offset of 5 ms BD Tone re Onset of NE Tone (ms)

Fig 7: Comparing NE tone evoked inhibition at the BEF (34 kHz) (*left columns*) and at a non-BEF (43 kHz) (*right column*) during dichotic paired tone stimulation. Significant change was said to occur when the evoked spike count/latency at two consecutive ISIs was <50% or 1 SD from the baseline response. *A*: Dot raster displays depicting spike suppression in a short-pass DTN in response to both conditions when the BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear. Spike suppression begun when the 5 ms BD tone and the 50 ms NE tone overlapped in time. The duration of spike suppression was 64.57 ms *B*: Mean ± SE spikes per stimulus as a function of the ISI between the BD and NE tones. Starting from the left, the shortest ISI in which the spike count was ≤ 50% of baseline was T_1 = 1 ms. The longest ISI, in which the spike count was ≤50% of baseline was T_2 = 75 ms. *C*: Mean ± SE FSL as a function of the ISI between the BD and NE tones. The shortest ISI in which the FSL deviated by >1 SD from baseline was T_1 = 1 ms, and the longest ISI in which the FSL deviated by >1 SD from baseline was T_2 = 67 ms. *D*: Mean ± SE LSL as a function of the ISI between the BD and NE tones. There were no ISIs in which the LSL deviated by >1 SD from the baseline. In the BEF condition, the earliest T_1 time was 1 ms and the latest T_2 time was 75 ms; T_1 was determined with the spike counts and/or FSL criteria while T_2 was determined with a spike count criterion. The latency of inhibition led the FSL by 2.57 ms, and the inhibition persisted 14.57 ms longer than the NE tone. *E*: Dot raster display depicting responses of the same DTN when the NE tone was at non-BEF. *F*: The shortest ISI in which the spike count fell to ≤ 50% of baseline was T_1 = 9 ms, and the longest ISI in which the spike count was ≤50% of baseline was T_2 = 27 ms. Changing the frequency of the NE tone away from the BEF resulted in a shortened duration of spike suppression (17.61 ms). There was no significant deviation in either the (G) FSL or (*H*) LSL of the cell. In the non-BEF condition, the earliest T_1 time was 9 ms, the latest T_2 time was 27 ms, and both were determined with spike counts. The latency of inhibition lagged behind the FSL by 4.39 ms, and the inhibition ended 32.39 ms prior to the offset of the NE tone. This example shows

evidence that ipsilaterally evoked inhibition at BEF can be weakened when the NE tone is presented at a non-BEF (0.34 octaves re BEF). n = 10 trials per stimulus.

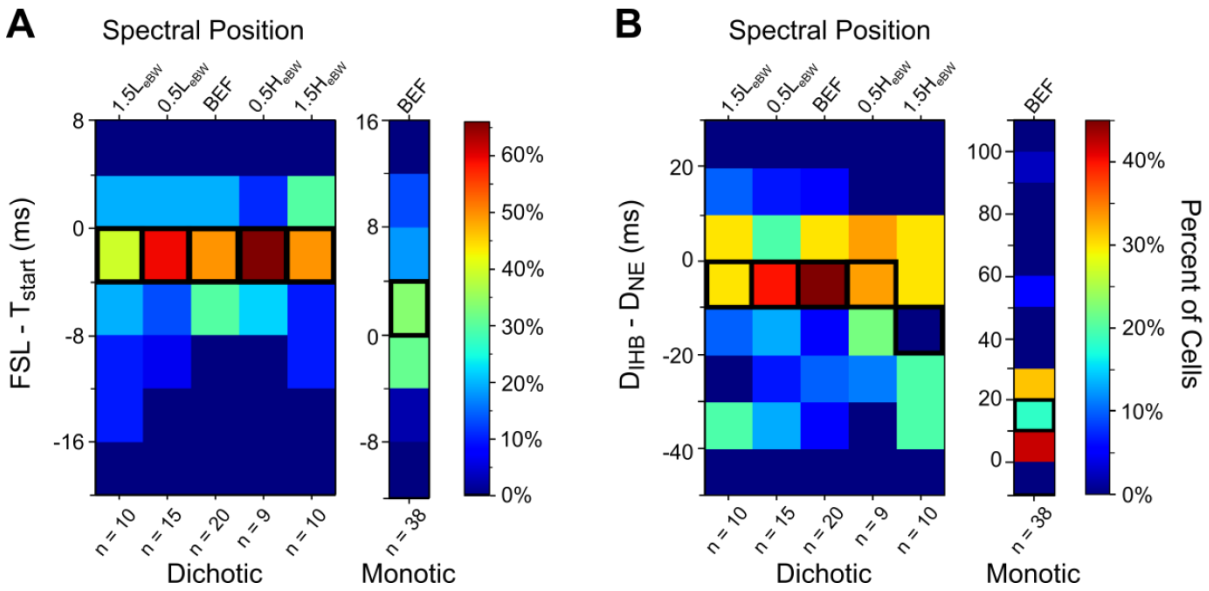
A
Spectral Position
1.5L$_{eBW}$
0.5L$_{eBW}$
BEF
0.5H$_{eBW}$
1.5H$_{eBW}$
FSL - T$_{start}$ (ms)
8
0
-8
-16
n = 10
n = 15
n = 20
n = 9
n = 10
Dichotic
16
8
0
-8
BEF
n = 38
Monotic
60%
50%
40%
30%
20%
10%
0%
B
Spectral Position
D$_{IHB}$ - D$_{NE}$ (ms)
20
0
-20
-40
100
80
60
40
20
0
40%
30%
20%
10%
0%
Percent of Cells

Fig 8: Two-dimensional histograms of the distribution of the duration of leading/lagging and persistent inhibition across the 5 standardized NE tone frequencies for DTNs tested with dichotic paired-tone stimulation (n = 64). All cells tested in the monotic condition (n = 38) when the BD tone frequency was equal to the NE tone frequency are displayed. Mean duration of inhibition at each NE tone frequency is indicated by a *black box*, with the colour scale showing the proportion of DTNs per bin. *A*: Distribution of the difference between the excitatory FSL and latency of inhibition (FSL - T_{start}) evoked by NE tones at 5 standardized frequencies. Cells with a positive difference have leading inhibition; cells with a negative difference have lagging inhibition. There was no difference in the distribution of FSL - T_{start} across the NE tone frequencies; however, there was a significant difference between the dichotic and monotic conditions at BEF. Moreover, most cells had leading inhibition (n = 24) in the monotic condition, but displayed lagging inhibition (n = 46) in the dichotic condition. *B*: Distribution of the difference between the duration of inhibition evoked by the NE tone and the duration of the NE tone stimulus (D_{IHB} - D_{NE}). Cells with a positive difference have persistent inhibition; cells with a negative difference have inhibition lasting less than the duration of the NE tone. The distribution of $D_{IHB} – D_{NE}$ was generally consistent across the dichotic condition, but greatly differed significantly from the monotic condition. All cells tested in the dichotic condition displayed no persistent inhibition, while the same cells tested in the monotic condition always had persistent inhibition (see Table 1). Number of DTNs tested = 10 ($1.5L_{eBW}$), 15 ($0.5L_{eBW}$), 20 (BEF), 9 ($0.5H_{eBW}$), 10 ($1.5H_{eBW}$), 38 (Monotic).

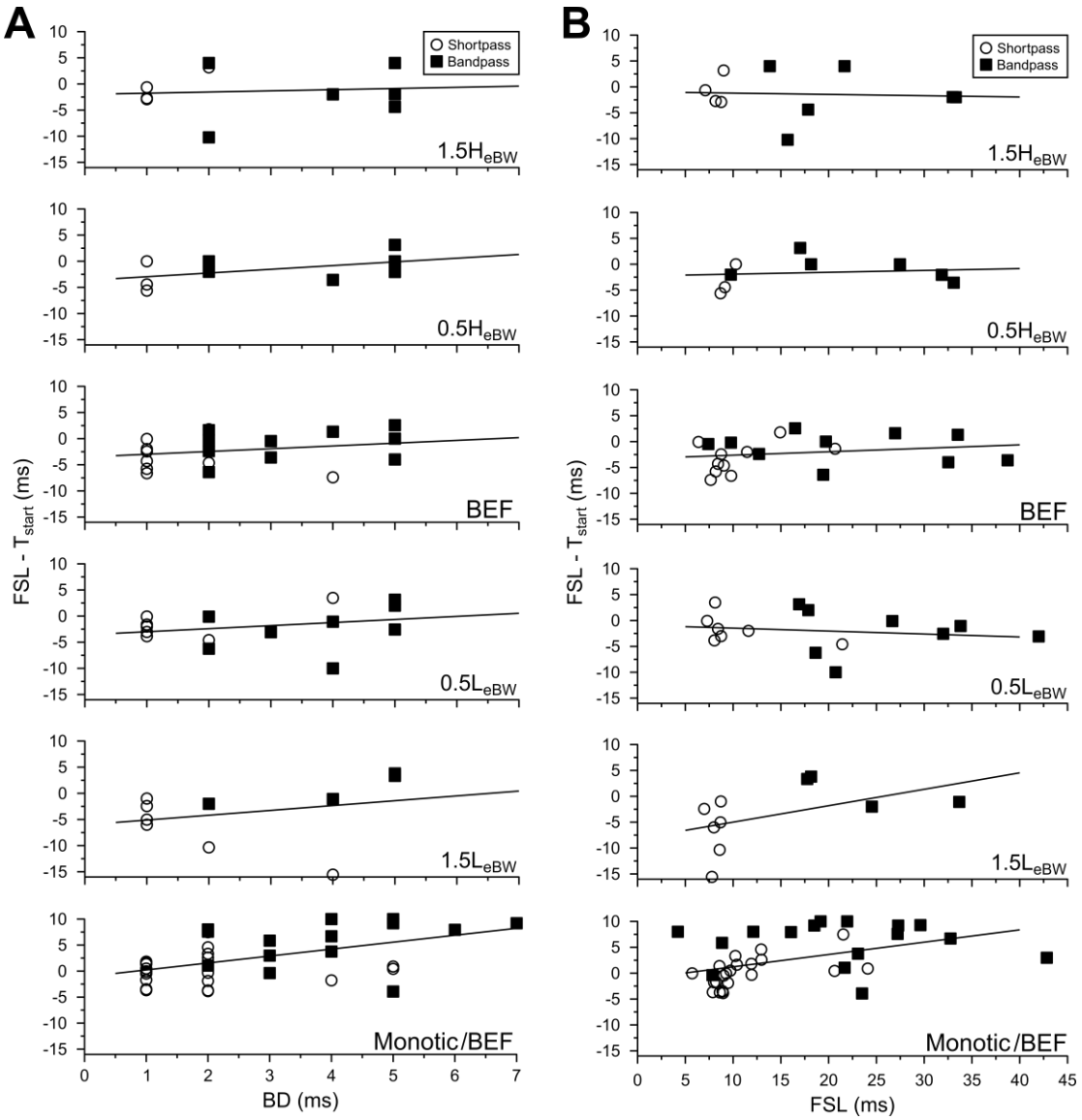
A
B
Shortpass
Bandpass
1.5H$_{eBW}$
0.5H$_{eBW}$
BEF
0.5L$_{eBW}$
1.5L$_{eBW}$
Monotic/BEF
FSL - T$_{start}$ (ms)
BD (ms)
FSL (ms)

Fig 9: Relations of the duration of leading/lagging inhibition to BD, FSL, and duration filter class. Duration of inhibition measured with paired-tone stimulation at 5 standardized NE tone frequencies in the dichotic condition and in the monotic condition at BEF. *A*: Duration of leading/lagging inhibition did not correlate to BD at any NE tone frequency in the dichotic condition; in the monotic condition the duration of leading inhibition increased in DTNs tuned to longer BDs. *B*: Duration of leading/lagging inhibition also did not correlate with FSL in the dichotic conditions at any NE tone frequency; however, in the monotic condition the duration of leading inhibition increased in DTNs with longer FSLs. There was no difference between duration filter classes in most dichotic conditions, with the exception being in the $1.5L_{eBW}$ condition band-pass DTNs exhibited longer amounts of leading inhibition when compared to short-pass DTNs. In the monotic condition, band-pass DTNs exhibited longer amounts of leading inhibition than short-pass DTNs. Sample sizes, regression equations, correlation coefficients (*R*), and *P* values for each linear regression are listed in Table 2. Mann-Whitney *U*-tests for the comparison between duration filter classes are listed in Table 3.

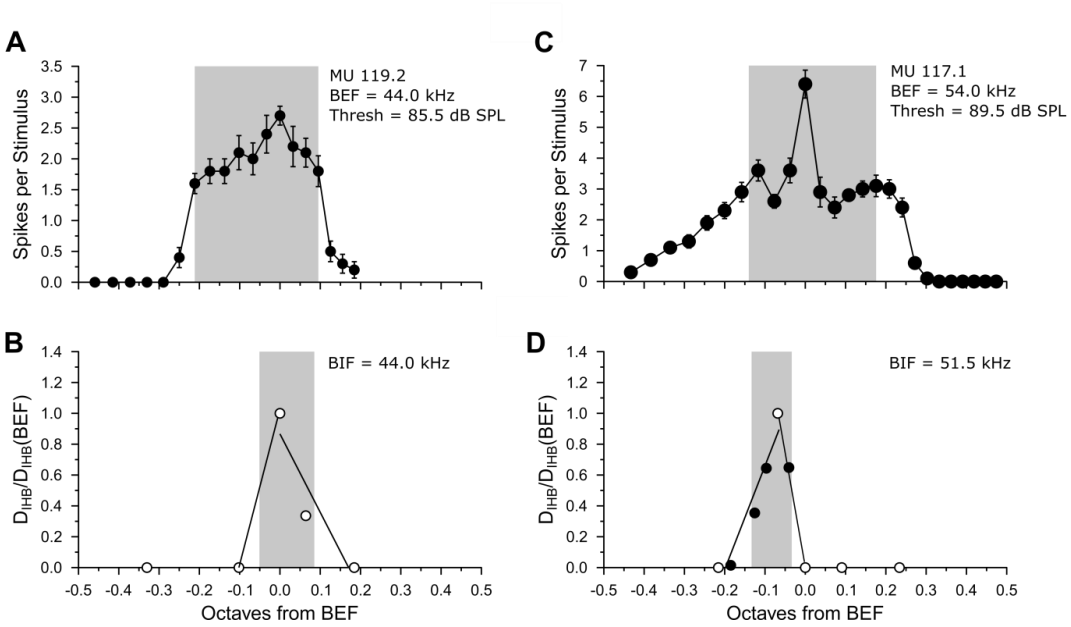
A
MU 119.2
BEF = 44.0 kHz
Thresh = 85.5 dB SPL
Spikes per Stimulus
C
MU 117.1
BEF = 54.0 kHz
Thresh = 89.5 dB SPL
Spikes per Stimulus
B
BIF = 44.0 kHz
DIHB/DIHB(BEF)
Octaves from BEF
D
BIF = 51.5 kHz
DIHB/DIHB(BEF)
Octaves from BEF

Fig 10: Excitatory and inhibitory best frequencies, FRAs, and BWs of DTNs. The *top* row displays the excitatory FRA of a DTN, plotted as the mean ± SD spike count as a function of BD tone frequency. The *bottom* row corresponds to the inhibitory FRA of the same DTN measured during binaural paired-tone stimulation, plotted as the normalized duration of inhibition, relative to BEF, in octaves as a function of NE frequency. *A*: The eFRA of a band-pass DTN with a BEF of 44.0 kHz and an eBW ranging from 38.0 to 47.0 kHz or -0.212 to 0.095 octaves (re BEF) (*grey box*). *B*: The normalized iFRA of the same DTN plotted as the duration of inhibition evoked at different NE tone frequencies. For this cell, the BIF was 44.0 kHz, and matched the BEF (44.0 kHz). The iBW ranged from 42.5 to 46.7 kHz and was narrower that the eBW (-0.051 to 0.085 octaves re BEF) (*gray box*). The five standardized NE tone frequencies are illustrated with *open circles*. Separate linear regressions were calculated for the low- and high-frequency slopes flanking the iFRA to measure the cell's iBW. *C*: The eFRA of a short-pass DTN with a BEF of 54.0 kHz, and a eBW ranging from 49.0 to 61.0 kHz or -0.139 to 0.176 octaves (re BEF). *D*: Normalized iFRA of the same cell with a BIF of 51.5 kHz, that is -0.068 octaves below the BEF, and an iBW of 49.2 to 52.8 kHz or -0.133 to -0.034 octaves (re BEF). In all cases, the last point included in the linear regression was the first data point to reach ≤0.1, starting from the BIF and moving lower/higher in frequency. Stimuli presented at +10 dB (re BEF, BD threshold); n = 10 trials per stimulus.

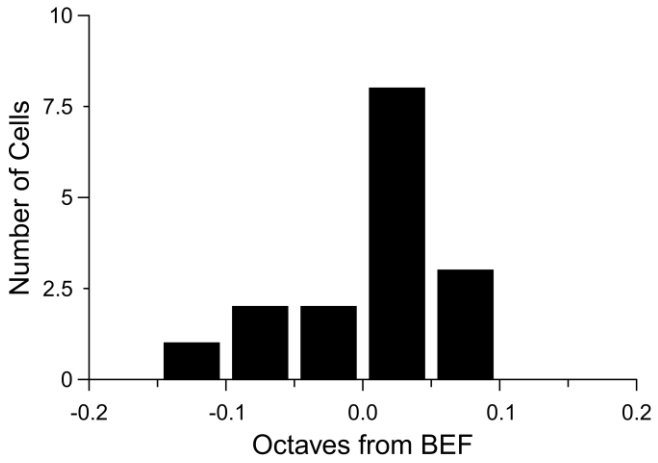

Fig 11: Histogram depicting the difference in octaves between the BEF and BIF in DTNs tested with dichotic binaural paired-tone stimulation. Positive values represent a higher BIF compared to the BEF, and negative values represent cells with a lower BIF compared to their BEF. The majority of DTNs had a very close match between the BEF and BIF, with the highest percentage of cells sharing the same value between the two conditions (7 cells, 43.75%). n = 16.

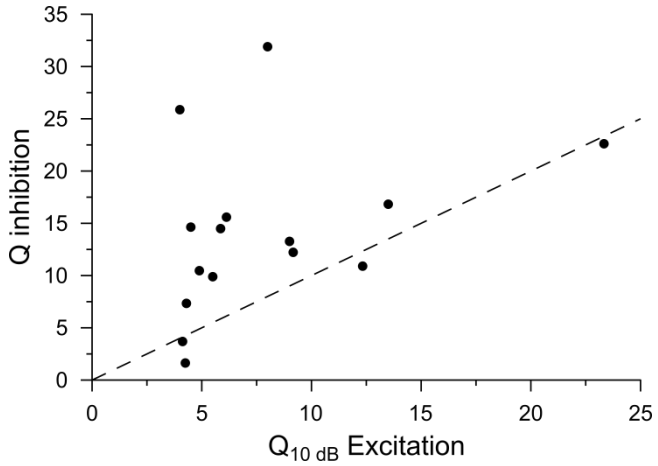

Fig 12: Excitatory and inhibitory tuning sharpness in DTNs tested with dichotic binaural paired-tone stimulation. Tuning sharpness plotted as a quality factor (Q) along with the identity line ($y = x$). Excitatory tuning sharpness measured as $Q_{10\ dB}$ = BEF/eBW, and inhibitory tuning sharpness measured as Q = BIF/iBW. Cells had inhibitory Q factors that were larger than the corresponding excitatory $Q_{10\ dB}$ factors in the same DTNs. This demonstrates that ipsilateral inhibition was more sharply tuned relative to contralaterally-evoked excitation. n = 16 cells.

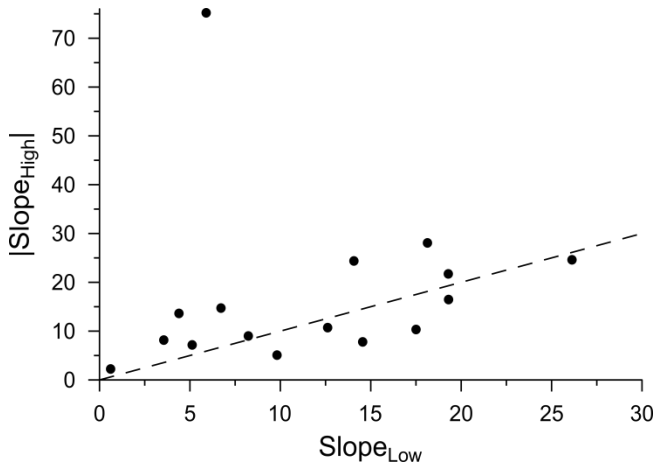

Fig 13: Low- and high-frequency tuning slope steepness measured from the normalized iFRA of DTNs tested with dichotic binaural paired-tone stimulation. Points above the $y = x$ identity line are cells that exhibited an iFRA with a steeper high-frequency slope than low-frequency slope, and points below the identity line indicate cells that exhibited an iFRA with a steeper low-frequency slope steeper than high-frequency slope. There was no difference in steepness between the flanking slopes. n = 16 cells.

Tables

Table 1. Proportion of DTNs showing leading and persistent inhibition at 5 standardized dichotic NE tone frequencies and in the monotic BEF condition, relative to each cell's eBW. Also shown are the results of Kruskal-Wallis post-hoc pairwise comparisons on the duration of leading and persistent inhibition across all conditions. Statistically significant correlations are indicated in **boldface**. n = 10 ($1.5L_{eBW}$), 15 ($0.5L_{eBW}$), 20 (BEF), 9 ($0.5H_{eBW}$), 10 ($1.5H_{eBW}$), 38 (monotic).

		Pairwise Comparisons of Duration of Leading Inhibition*					
	% DTNs with Leading Inhibition	Monotic	$1.5L_{eBW}$	$0.5L_{eBW}$	BEF	$0.5H_{eBW}$	$1.5H_{eBW}$
Monotic	63%		**0.002**	**0.039**	**0.004**	**0.023**	**0.041**
$1.5L_{eBW}$	20%						
$0.5L_{eBW}$	20%		0.675				
BEF	25%		0.839	0.786			
$0.5H_{eBW}$	11%		0.589	0.855	0.673		
$1.5H_{eBW}$	30%		0.419	0.641	0.465	0.805	
		Pairwise Comparisons of Duration of Persistent Inhibition*					
	% DTNs with Persistent Inhibition	Monotic	$1.5L_{eBW}$	$0.5L_{eBW}$	BEF	$0.5H_{eBW}$	$1.5H_{eBW}$
Monotic	100%		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
$1.5L_{eBW}$	40%						
$0.5L_{eBW}$	27%		0.850				
BEF	35%		0.990	0.810			
$0.5H_{eBW}$	33%		0.840	0.971	0.808		
$1.5H_{eBW}$	30%		0.566	0.660	0.499	0.721	

* significant differences determined with Kruskal-Wallis post-hoc pairwise comparisons

Table 2. Linear relationships on the duration of leading inhibition ($L_{first} - T_{start}$) and BD or FSL for DTNs tested with monotic and dichotic paired-tone stimulation. Statistically significant correlations are indicated in **boldface**. The only significant correlation was in the monotic condition when the BD and NE tones were presented monaurally and both tones were matched to the BEF.

Comparison	Frequency	N	Slope	Intercept	*R*	*P* Value
$L_{first} - T_{start}$ (ms)	Monotic	38	1.3328	-1.104	0.496	**0.002**
vs. BD (ms)	$1.5L_{eBW}$	10	0.9237	-6.032	0.267	0.456
	$0.5L_{eBW}$	15	0.5882	-3.5812	0.27	0.333
	BEF	20	0.5309	-3.521	0.25	0.287
	$0.5H_{eBW}$	9	0.7105	-3.668	0.482	0.189
	$1.5H_{eBW}$	10	0.2242	-1.998	0.09	0.805
$L_{first} - T_{start}$ (ms)						
vs. FSL (ms)	Monotic	38	0.2378	-1.148	0.46	**0.004**
	$1.5L_{eBW}$	10	0.3174	-8.164	0.485	0.156
	$0.5L_{eBW}$	15	-0.0568	-0.905	0.174	0.536
	BEF	20	0.067	-3.301	0.217	0.359
	$0.5H_{eBW}$	9	0.0356	-2.269	0.132	0.736
	$1.5H_{eBW}$	10	-0.0249	-0.952	0.056	0.878

Table 3. Results of Mann-Whitney *U*-tests comparing the duration of leading inhibition ($L_{first} - T_{start}$) in short-pass and band-pass DTNs at monotic and 5 dichotic standardized NE tone frequencies relative to each cell's 50% excitatory bandwidth. Statistically significant differences are indicated in boldface. n = 10 ($1.5L_{eBW}$), 15 ($0.5L_{eBW}$), 20 (BEF), 9 ($0.5H_{eBW}$), 10 ($1.5H_{eBW}$), 38 (monotic)

Condition	Duration Filter Class	Mean ± SD	*U*	*P* Value
Monotic	Short-pass	0.01 ± 2.93	49.000	**0.000**
	Band-pass	5.95 ± 4.13		
Dichotic	Duration Filter Class	Mean ± SD	*U*	*P* Value
$1.5L_{eBW}$	Short-pass	-6.73 ± 5.40	2.000	**0.038**
	Band-pass	1.02 ± 2.98		
$0.5L_{eBW}$	Short-pass	-1.67 ± 2.71	27.000	0.955
	Band-pass	-2.24 ± 4.31		
BEF	Short-pass	-3.28 ± 2.96	29.000	0.123
	Band-pass	-1.16 ± 2.86		
$0.5H_{eBW}$	Short-pass	-3.35 ± 2.96	4.000	0.262
	Band-pass	-0.75 ± 2.34		
$1.5H_{eBW}$	Short-pass	-0.78 ± 2.83	12.000	1.000
	Band-pass	-1.76 ± 5.39		