DICHOTIC RESPONSE PROPERTIES

OF DURATION-TUNED NEURONS IN THE BAT

Response Properties of Ipsilateral Inputs in Duration Tuned Neurons

by

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Abstract

Inhibitory synaptic inputs are necessary for the formation of duration tuned neurons (DTNs) in the mammalian inferior colliculus (IC). These neurons may help to differentiate the temporal aspects of sound. Much more is known about the characteristics of the monaural contralateral inputs that create DTNs compared to their ipsilateral inputs. The current study used paired-tone stimulation and single-unit extracellular recording to measure the frequency tuning of the neural inhibition evoked from stimulating the ipsilateral ear in the big brown bat (*Eptesicus fuscus*). The stimulus consisted of a short duration, excitatory probe tone set to the cell's best duration (BD) and varying in onset time relative to a longer duration, non-excitatory (NE) suppression tone. 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 and the NE tone was presented to the ipsilateral ear (i.e. the same side as the IC hemisphere being recorded), but now the NE tone suppressed BD tone evoked spiking in a smaller proportion of cells. Frequency tuning, latency, and duration of the ipsilaterally-evoked inhibition was investigated by varying the NE tone frequency both within and outside a cell's 50% excitatory bandwidth (eBW) that was measured by stimulating the contralateral ear only. The inhibition evoked contralaterally differed from that evoked ipsilaterally in three respects: 1) the contralateral inhibition led contralateral excitation whereas ipsilateral inhibition usually lagged contralateral excitation; 2) the duration of contralateral inhibition persisted as long or longer than the duration of the NE tone, whereas the duration of ipsilateral inhibition was usually shorter than the NE tone duration; and 3) the

bandwidth of contralateral inhibition was broader than the cell's eBW, while the bandwidth of ipsilateral inhibition was more variable compared to the eBW. There were some similarities between the contralateral and ipsilateral evoked inhibitions: 1) the best inhibitory frequency (BIF) and best excitatory frequency (BEF) matched in both conditions; 2) the duration of inhibition decreased as the NE tone frequency departed from the cell's BEF; and 3) the onset of inhibition was frequency independent both in the monotic and dichotic conditions. These data suggest the temporal selectivity of midbrain DTNs is created by monaural auditory inputs, with ipsilateral inputs playing a lesser role in shaping the cell's duration selectivity. Ipsilateral inhibition, when present, may play a role in shaping the response of DTNs to sounds presented in different locations rather than creating duration-selectivity like the monaural pathway.

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BD - best duration

BEF - best excitatory frequency

BIF - best inhibitory frequency

D_{BD} - duration of BD tone

D_{IHB} - duration of inhibition

D_{NE} - duration of NE tone

DTN - duration-tuned neuron

EE – excitatory contralaterally, excitatory ipsilaterally

EI - excitatory contralaterally, inhibitory ipsilaterally

EO - excitatory contralaterally, no input ipsilaterally

eBW - excitatory bandwidth

eFRA - excitatory frequency response area

FSL - first spike latency

H_{eBW} - high frequency partition of eBW

iBW – inhibitory bandwidth

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

In aquatic and terrestrial environments, sound is ubiquitous and can help individuals localize and communicate with other individuals within their environment. Sounds can be described by three physical attributes: amplitude, frequency, and time. These attributes would subjectively be known as loudness, pitch, and distance, respectively. The central nervous system (CNS) of many animals have developed complex neural circuits to recognize and discriminate different attributes of sound. The central auditory system then transmits this sensory information to higher-level brain structures that can affect behavior (Chiel and Beer, 1997).

The duration of a stimulus is an important aspect of sound that can provide differentiating information to the organism playing an important role in the recognition of conspecific signals (Narins and Capranica, 1980), predatory calls (Ehrlich et al., 1997), and speech recognition in humans (Denes, 1955; Shannon et al., 1995). Duration also plays a crucial role for echolocating bats, who use biosonar for navigation and foraging. Some bats can detect small differences between echolocation calls that allow them to differentiate between objects whose distances differ by 1 mm (Neuweiler, 1990).

During echolocation, a bat will typically emit a series of calls that reflect off objects as echoes. When bats are initially searching for prey, they emit long, quasi-constant frequency calls at a low repetition rate. Bats are likely using low frequency calls to avoid the effects of atmospheric attenuation, which affects how far both calls and subsequent echoes travel through the air. After a prey item is detected, bats switch their calls to shorter, broad-band frequency calls. If the target (e.g. an insect) is smaller than the smallest wavelength emitted by the bat, it will not reflect a substantial echo. The

increase in call bandwidth by the bat is likely an adaption to increase the resolution of echoes returning to the bat. During the approach and capture phases of echolocation, the calls emitted by the bat are narrower in bandwidth, shorter in duration, and at a higher repetition rate. The entire process starts again from the beginning immediately after an insect is captured (Au et al., 1988; Schnitzler and Kalko, 2001).

Bats also have highly specialized sensory adaptions to detect slight differences in time and frequency between outgoing calls and returning echoes. For temporal discriminations on the order of milliseconds to be achieved, the CNS would require cellular mechanisms with the ability to precisely differentiate signal durations. In the inferior colliculus (IC) of bats, as well as other mammals, there exists a class of neurons whose spiking responses are selective to specific durations called duration tuned neurons (DTNs). Each DTN has a different best duration (BD) stimulus that elicits maximal spiking. Like all auditory neurons, DTNs are also selective for stimulus frequency and amplitude (Morrison et al., 2014). Though DTNs have been studied mainly in bats and their responses are likely important for echolocation, they have also been found in the auditory midbrain of a variety of taxa including rats (Perez-Gonzalez et al. 2006), mice (Brand et al. 2000; Xia et al. 2000), guinea pigs (Wang et al. 2006) and chinchillas (Chen 1998). The duration selective properties of DTNs are known to be a result of interacting inhibitory and excitatory synaptic inputs offset in time (for a review, see Fuzessery & Hall, 1999; Sayegh et al., 2011). These cells are not restricted to audition, as DTNs have also been reported from the visual cortex of cats (Duysens et al., 1996). Because DTNs are found in both echolocating and non-echolocating vertebrates, across several classes of

vertebrates, and in different sensory modalities, they likely serve a crucial role in general sensory perception.

Classes of DTNs can be further divided by their unique response properties across stimulus durations. A DTN's BD is determined by presenting a range of durations while holding stimulus frequency and amplitude constant. Typically, the stimulus frequency used is that which elicits the greatest number of spikes – called the best excitatory frequency (BEF) – while the stimulus amplitude is set to +10 dB above the cell's excitatory threshold. At least three different types of DTNs have been classified based on their unique spiking response characteristics (Ehrlich et al., 1997; Jen and Zhou, 1999; Sayegh et al., 2011): (1) Short-pass DTNs have the greatest spike count at or below their BD, with a reduction in spiking (to <50% of BD) at longer durations; (2) Band-pass DTNs also have their greatest spike count at BD, with a reduction in spiking (to <50% of BD) at longer and shorter durations; (3) Long-pass DTNs do not have a BD but instead continue spiking to all durations above a minimum duration. The exact role of duration-tuning is still not fully known, but several hypotheses have been presented to explain their potential role (Casseday et al., 1994; Chiel and Beer, 1997).

Neurons with duration selectivity were first found in the inferior colliculus and are thought to be created there (Potter, 1965; Casseday et al., 1994, 2000; Covey et al., 1996). Neuropharmacological studies with monaural (contralateral ear) stimulation have shown that inhibition is necessary for the formation of DTNs, that this inhibition persists longer than the duration of the stimulus that evoked it, and that it arrives before excitation (Casseday et al., 1994; Faure et al., 2003). Both ipsilateral and contralateral inputs are present in some DTNs and may play a role in duration selectivity; however, the neural

mechanisms underlying duration-tuning are not fully understood. Approximately 50% of DTNs receive ipsilaterally-evoked inhibition, though this inhibition is shorter in duration and longer in latency than the contralateral inhibition that creates duration selectivity (Sayegh et al., 2014). Studies measuring the spectral tuning of the contralaterally-evoked inhibitory inputs to DTNs have found that the onset of inhibition was independent of stimulus frequency, but the offset and duration of inhibition systematically decreased as stimulus frequency deviated from the DTN's BEF (Valdizón-Rodríguez and Faure, 2017). The same study also found that the BEF closely matched the best inhibitory frequency (BIF), but that the excitatory bandwidth (eBW) was more narrowly tuned than the inhibitory bandwidth (iBW). It is not known if the frequency tuning of the ipsilateral inhibition differs from that of the contralateral inhibition.

The goal of the current study was to further characterize the frequency-tuning properties of the ipsilaterally-evoked inhibition to DTNs using monotic and dichotic paired-tone stimulation in a manner similar to Valdizon-Rodriguez and Faure (2017). The stimulation paradigm involves varying the onset of an excitatory, pure tone stimulus set to a cell's BD and BEF against a stationary, non-excitatory tone duration that was varied in frequency. A baseline spike count for the excitatory BD tone was used for determining stimulus frequencies that evoked ipsilateral inhibition. I found that inhibition preceded excitation contralaterally but followed excitation ipsilaterally. The duration and offset of the inhibitions contralaterally and ipsilaterally-evoked were frequency dependent, while the onset of inhibition was frequency independent. These results confirm that DTNs are formed through monaural neural circuits and these responses may be modulated in some cells by binaural inputs.

Materials and Methods

Surgical preparation

Electrophysiological recordings were obtained from the IC of 14 big brown bats (*Eptesicus fuscus*; 4 males, 10 female). To ensure stable recordings, each bat underwent a preparatory surgery where a stainless steel post was attached to the dorsal surface of the skull to immobilize the bat's head. Prior to the surgery, the bat was transported from a holding room to the surgery room and given a subcutaneous (SQ) injection of the opioid buprenorphine (~0.03 mL Temgesic; 0.045 mg/kg), to prevent any discomfort experienced during and following the surgery. The bat was then placed into an (12 x 10 x 10 cm) anesthesia induction chamber to breathe an isoflurane and oxygen mixture (1-5% volume/volume) at a rate of 1 L/min until the animal was fully anesthetized (~ 8 minutes). Breathing was closely monitored during induction. Once fully anesthetized, the bat was transferred to a custom stereotaxic frame and its mouth was placed in a custom bite-bar that stabilized the head during surgery and that functioned as gas mask to deliver isoflurane anesthesia. The fur on the dorsal surface of the head was cleaned with povidone-iodine surgical scrub (Betadine™) before being cropped. Then a midline incision was made in the scalp with a #10 scalpel. Small portions of skin were removed and/or reflected to expose the musculature beneath. A local anaesthetic (~0.2 mL of Bupivacaine SQ, 5 mg/mL) was applied superficially to the surrounding muscle tissue. The temporal muscles were then reflected, and the skull underneath was scraped and cleaned with 70-100% ethanol. Once dry, a stainless-steel post was affixed to the skull with cyanoacrylate adhesive and liquid hardener (Pacer Zipkicker). One end of a chloride silver electrode wire that was attached to the head post was placed under the temporal

muscles and served as a reference electrode. Following surgery, the wound was covered with gelfoam coated with Polysporin™. The bat was then placed in a stainless steel holding cage to recover and rest for 1-3 days before recording commenced.

Electrophysiological recordings

The head of the bat was immobilized by attaching the distal end of the head post to a customized holder mounted on a stereotaxic micromanipulator (David Kopf Instruments Model 9000). Recordings took place inside a double-walled, sound attenuation booth with electrical shielding (Industrial Acoustics Co., Inc). A neuroleptic (0.3 mL Innovar-Vet; 1:1 v/v mixture of 0.25 mg/mL of fentanyl citrate and 1.25 mg/mL droperidol; 9.6 mg/kg) was administered subcutaneously, lightly sedating the animal. The sedated animal was then placed in a foam-lined body restraint that was suspended by springs within a small animal stereotaxic frame (ASI Instruments). The entire apparatus rested on top of an air vibration table (TMC Micro-G).

In the bat, the IC's can be detected as two white ellipses below the lambdoid suture. Each IC was exposed through a craniotomy using a no. 11 scalpel. The dura mater was carefully removed from the surface of the IC with a sharp pin along with any potential debris that may have fallen near the craniotomy. Single-unit extracellular recordings were conducted with thin wall borosilicate glass microelectrodes (outside diameter = 1.2 mm; A-M Systems Inc.) filled with 3M NaCL. The electrode was manually positioned directly above the craniotomy and lowered into the brain with a stepping hydraulic micropositioner (Kopf Model 2650). Electrode resistances typically ranged between 10 - 30 MΩ. Action potentials were recorded with a neuroprobe

amplifier (Model 1600; A-M Systems Inc.) whose 10x output was bandpass filtered and further amplified (500 - 1000x) by a Tucker Davis Technologies (TDT) spike conditioner (TDT PC1; lowpass fc= 7 kHz; highpass fc= 300 Hz). Spike times were logged on to a computer by passing the PC1 output to a spike discriminator (TDT SD1) and then an event timer (TDT ET1) synchronized to a timing generator (TDT TG6). Recording sessions took place in 6-8 hour periods, with up to 8 recording sessions per bat, and were terminated if there were any signs of discomfort during recording.

Stimulus generation and data collection

Custom software was used for stimulus generation and data collection. Spike-times were displayed as dot rasters ordered by the acoustic parameter that was varied. The acoustic stimulus was digitally generated with a two-channel array processor (TDT Apos II; 357 kHz sampling rate) 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: fc = 120 kHz), two programmable attenuators (TDT PA5), and two signal mixers (TDT SM5) with equal weighting. The output of each mixer was rooted through a manual attenuator (Leader LAT-45) before final amplification (Krohn-Hite Model 7500) and transduction by one of two Brüel & Kjær (B&K) ¼-inch condenser microphones (Type 4939; protective grid on) modified for use as loudspeakers with a transmission adaptor (B&K Type UA-9020) to correct for nonlinearities in the transfer function. The microphones were positioned ~1 mm in front of the external auditory meatus. The output of each speaker was measured and calibrated with a B&K Type 4138 ⅛-inch condenser microphone (90° incidence; grid off) connected to a measuring amplifier (B&K Type

2606), bandpass filter (K-H Model 3500), and referenced to a sound calibrator (B&K Type 4231) expressed in decibels sound pressure level (dB SPL re 20 μPa) equal to the peak amplitude of continuous tones of the same frequency. The loudspeaker transfer function was flat ± 6 dB from 28 to 118 kHz, and attenuation was at least 30 dB at the ear opposite of the source (Ehrlich et al., 1997). Acoustic pulses had a rise/fall time of 0.4 ms, were shaped with square cosine function, and presented at a rate of 3 Hz.

Paired tone stimulation

Stimulus paradigms for monaural and binaural paired-tone stimulation were used as described by Faure et al. (2003), Sayegh et al. (2014), and Valdizon-Rodriguez and Faure (2017).

Monaural (monotic) 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. The basic paradigm involves stimulating a cell with two pure tone pulses that 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 longer, non-excitatory duration that was ten times the duration of the BD tone to ensure a constant energy relationship between the two signals, regardless of the cell's BD. The NE tone was initially set to the BEF. The onset time of the NE tone was fixed between stimulus presentations, whereas the onset time of the BD tone was randomly varied (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. Because the BD tone evoked maximal spiking, any changes in spiking were caused by presentation of the NE tone.

Binaural (dichotic) paired-tone stimulation used the same stimulation parameters as monaural (monotic) paired-tone stimulation with the following exceptions: 1) the NE tone was broadcasted to the ipsilateral ear; and 2) the NE tone frequency varied relative to the BEF. When the two tones were not matched in frequency, when they overlapped 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.

I tested DTNs with dichotic, binaural paired-tone stimulation at 5 NE tone frequencies standardized to the BEF and eBW of each cell (Fig. 1). Using monaural and binaural paired-tone stimulation, I measured and compared the inhibition evoked by the NE tone through the contralateral and ipsilateral central auditory pathways. A cell's eBW was divided into lower (L_{eBW}) and higher (H_{eBW}) spectral partitions re BEF. The 5 standardized frequencies were selected as: (1) 1.5 times below the L_{eBW} cut-off frequency ($1.5L_{eBW}$); (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} cut-off frequency ($1.5H_{eBW}$). Thus, each cell was tested with at least three NE tone frequencies within the 50% eBW ($0.5L_{eBW}$, BEF, $0.5H_{eBW}$) and two NE tone frequencies outside the 50% eBW ($1.5L_{eBW}$, $1.5H_{eBW}$). Whenever possible, additional NE tone frequencies were also tested.

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 spike latencies. This method was established by Sayegh et al., (2014) and has been used to measure the frequency tuning of contralateral inhibition with monaural paired-tone stimulation (Valdizon-Rodriguez and Faure, 2017). In both monotic and dichotic paired-tone stimulation, the baseline response of a cell was quantified at the 10 longest ISIs when the BD tone preceded the NE tone to ensure that the cell's spiking was not affected by the NE tone-evoked inhibition. This was done to all but 3 cell's where less than 10 ISIs were used for baseline firing measurement. The mean $\pm$ standard deviation (SD) baseline spike count, first spike latency (FSL), and last spike latency (LSL) were calculated for each cell from these 10 responses.

Using three criteria, I compared a cell's baseline responses with those obtained at other ISIs to determine the time points when spikes were suppressed and/or altered in latency by NE tone-evoked inhibition. A 50% change in the evoked spike count (re baseline spike count) was the initial criterion used to determine the time points demarking the onset and offset of spike suppression. When possible, a 1 SD change in the evoked FSL and LSL (re baseline latencies) were used to refine and extend the initial estimate because changes in spike latency can result in a more sensitive measure of an altered response compared to changes in spike count (e.g. Faure et al., 2001; Sayegh et al., 2014; Valdizon-Rodriguez & Faure, 2017). Typically changes in LSL were used to

detect the onset of inhibition while changes in FSL were used to detect the offset of inhibition (re baseline latencies).

Each criterion yielded a pair of ISIs, T_1 and T_2, when BD tone evoked spikes were suppressed and/or altered in latency. From these I determined the onset (T_1) and offset (T_2) of the changed 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 of T_1 and T_2 were chosen using the most sensitive criterion that reflected the time course of the inhibition evoked by the NE tone using changes in the BD tone evoked spike count and/or spike latency. 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 accurate 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.

Calculating the latency and duration of NE tone evoked inhibition

After determining the final values of T_1 and T_2, I calculated the effective start time (T_{start}), end time (T_{end}), and duration of inhibition (D_{IHB}) evoked by the NE tone with:

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

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

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

where L_{last} was the baseline LSL, L_{first} was the baseline FSL, and D_{BD} was the duration of the BD tone, T_1 was the onset and T_2 was the offset of the inhibition evoked by the NE tone. Because the paired-tone stimulation paradigm uses a roving BD tone and a stationary NE tone, any changes that occur in spiking are a result of 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.

The average number of spikes per stimulus and the average FSL and LSL were calculated off-line using custom software with an analysis window tailored to the response of each cell. The start and stop times of the analysis window, which were anchored to the onset and offset of the BD tone, were chosen to accommodate the latency and spike burst duration of each cell and to minimize the effects of spontaneous activity, if present.

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 the 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. $D_{IHB} > D_{NE}$). If inhibition did not persist longer than the NE tone, I am referring to it as intermittent inhibition (i.e. $D_{IHB} < D_{NE}$).

Calculating the eFRA, iFRA, BIF and iBW, and Tuning Sharpness

Initially, a cell's BD, BEF, and acoustic threshold were measured through the stimulation of the contralateral ear. I presented different frequencies (1 kHz steps) to each cell and formed an excitatory frequency response area (eFRA). The frequency that had the greatest amount of spiking was determined to be the BEF. Frequencies that elicited at least 50% of BEF firing were determined to be within the excitatory bandwidth (eBW) of the cell. I determined the best inhibitory frequency (BIF) and inhibitory bandwidth (iBW)

of a DTN using the changes in the pattern of spike suppression elicited from the BD-NE tone interaction. First, a cell's normalized iso-level inhibitory frequency response area (iFRA) was obtained at + 10 dB above the excitatory threshold. This was done by normalizing the D_{IHB} at each of the 5 standardized NE tone frequencies relative to the duration of inhibition evoked at the BEF and plotting this as a function of the NE tone frequency in octaves re BEF (Fig. 2). The BIF of a DTN was defined as the frequency with the largest normalized D_{IHB}. To compare the BIF to the BEF, each were converted to octaves (re BEF) and the difference of $\log_2$(BIF) - $\log_2$(BEF) was obtained for each cell. The iBW was estimated using at least 3 standardized frequencies and with at least one data point evoking inhibition. For estimating the slopes of the high and low frequency flanks of each cell's inhibitory tuning curve, both slopes included the BIF. I then used the low ($Slope_{Low}$) and high ($Slope_{High}$) frequency slopes of a cell's inhibitory tuning curve (re BIF) were used to define the lowest and highest cut-off frequencies of the iBW at 50% of the D_{IHB} obtained at the BIF. Slopes that were greater than two standard deviations away from the mean were excluded. For these regressions, the highest and lowest frequencies included were the first normalized data points that were less than 10% of the BIF D_{IHB}. A tuning quality (Q) factor, defined as Q = BIF/iBW for inhibition and Q_{10dB} = BEF/eBW for excitation, was calculated to report the sharpness of inhibitory tuning at +10 dB (re BD, BEF threshold) and excitatory tuning calculated from the 50% point of each cell's eFRA and reported at +10 dB above threshold. The two were then compared determine whether inhibition or excitation had sharper tuning. Because iBWs were normalized relative to inhibition evoked at the BEF, there are points that can exceed the value of 1 (or 100% of inhibition evoked at BEF).

Classification of Binaural Response Properties

To determine if a cell was EI or E0, I stimulated the contralateral ear with a BD, BEF tone at a constant amplitude (+10 dB re threshold), and presented the ipsilateral ear a tone that was varied in amplitude re: contralateral ear (Shen et al., 1997). A cell was defined to have ipsilateral inhibition (EI) if spiking was reduced to <50% of the original contralateral baseline firing rate. If a decrease in spike count was observed, then the cell was contributing ipsilateral inhibitory inputs to the DTN circuit. If little or no difference in spiking was seen from the initial contralateral recording, then there is no input evoked ipsilaterally to this circuit and is classified as E0.

I randomly varied the amplitude of a tone presented only to the ipsilateral ear at the cell's BD and BEF to measure the cell's ipsilateral rate-level function. The ipsilateral rate-level function was then compared to the contralateral rate-level function. A cell was defined as EE if it received excitatory inputs from both the contralateral and ipsilateral ears. A cell was said to have ipsilateral excitation when the peak firing rate of the ipsilateral rate-level function was > 50% of the contralateral rate-level function. If no excitation was recorded, this excluded the cell from the EE category and further testing commenced.

I assumed acoustic cross-over to begin when the amplitude disparity between the contralateral and ipsilateral ears was >25-30 dB SPL (Coles and Aitkin, 1979; Ehrlich et al., 1997). Therefore, I used a 25 dB maximum disparity to define EE, EI and EO cells because of acoustic cross-over previously defined. For example, if the initial contralateral acoustic threshold was 20 dB SPL and an increase in spiking was observed when the

ipsilateral tone was 45 dB SPL (keeping contralateral constant), this cell could have been affected by ipsilateral acoustic cross-over and was not considered an EI cell.

Data Analysis

Except for a cell's baseline response data, all data are reported as the mean $\pm$ standard error (SE). Each dataset was tested for normality and homogeneity of variances with Shapiro-Wilk's and Bartlett tests, respectively. When data did not meet the assumption of being normally distributed and/or equal in variance, equivalent non-parametric statistical tests were used. Sometimes cells could not be recorded through the entire experimental protocol, leading to unequal sample sizes between some of the 5 standardized frequencies ($1.5L_{eBW}$ = 33 cells, $0.5L_{eBW}$ = 35 cells, BEF = 42 cells, $0.5H_{eBW}$= 32 cells, $1.5H_{eBW}$ = 31 cells). When a DTN did not have measurable inhibition during dichotic paired-tone stimulation at any of the 5 standardized NE tone frequencies, this cell was excluded from independent-measures statistical analyses; however, if a cell displayed measurable inhibition at one of the 5 standardized frequencies, then data were included. Cells excluded from formal statistical analysis were represented in figures, tables, and summary statistics.

A Kruskal-Wallis test compared the proportion of DTNs that exhibited leading and persistent inhibition at each NE tone frequency. A Chi-squared test compared differences in duration of inhibition at each NE tone frequency. Mann-Whitney U-tests compared the duration of inhibition between short-pass and band-pass DTNs. The latency and duration of the inhibition evoked during monotic and dichotic paired-tone stimulation at the BEF 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 of each cell's iFRA. Linear regressions were used to calculate the relation of the duration of inhibition and BD and FSL at the 5 standardized NE tone frequencies and to calculate the low ($Slope_{Low}$) and high-frequency tuning slopes ($Slope_{High}$) of each cell's iFRA. All statistical tests were performed in SPSS and used an experiment-wise error rate of $\alpha = 0.05$.

Results

Monotic and dichotic paired-tone stimulation responses at the BEF

All cells in my sample had differing responses when paired-tone stimulation was presented in the monotic and dichotic conditions. I am going to present two examples of cells tested with monotic and dichotic paired-tone stimulation with both tones set to the cell's BEF and at equal amplitudes (+10 dB re BD threshold). The first example illustrates a neuron that showed measurable inhibition in both the monotic and dichotic conditions at the BEF (Fig. 4). The second example illustrates a neuron that had measurable inhibition in the monotic but no measurable inhibition when tested dichotically (Fig. 5). The first example cell was a band-pass DTN that had measurable inhibition monotically (Fig. 4A). The BD and NE tones were both set to the cell's BEF and were presented at an amplitude of +10 dB re BD tone threshold (i.e. 34 kHz and 60.5 dB SPL, respectively). In the monotic condition the cell displayed prominent inhibition evoked from the contralateral ear (67.55 ms). Spike counts were significantly reduced (Fig. 4B) and deviations in both the FSL and LSL were observed (Fig. 4, C and D), each providing the same value for the onset (T_1= -12) and (T_2= 56 ms). In the monotic

condition the cell displayed leading inhibition because inhibition arrived to the cell 15.55 ms sooner than the cell's excitatory FSL (L_{First} = 29.34 ms and T_{start} = 13.79 ms). The amount of leading inhibition observed in this DTN was much longer than other cells tested in the monotic condition. Because the duration of inhibition (67.55 ms) was longer than the NE tone duration (40 ms), the cell was said to exhibit persistent inhibition in the monotic condition. Spikes remained deviated/suppressed for 27.55 ms longer than the duration of the NE tone. The amount of persistent inhibition observed in this DTN was much longer than other cells tested in the monotic condition.

In the dichotic condition (Fig. 4E), the BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear. In this example cell, neural inhibition was present in the dichotic condition (Fig. 4E) resulting in a reduction of the cell's spike count (Fig. 4F) and deviations in both the FSL and LSL (Fig. 4 G and H). Inhibition was reduced, both in strength and duration, compared to baseline measurement of the first 7 positive ISIs in this cell. The values of T_1 and T_2 measured during dichotic paired-tone stimulation were 4 ms and 18 ms, respectively. The binaural condition displayed lagging inhibition, with inhibition arriving to the cell 0.29 ms after than the cell's excitatory FSL (L_{First} = 27.45 ms and T_{start} = 27.74 ms). Inhibition did not persist longer than the NE tone dichotically as the duration of inhibition was 13.71 ms shorter than the duration of the 40-ms NE tone.

In both the monotic and dichotic conditions, the cell displayed measurable inhibition that could be detected using changes in the evoked spike count, FSL, or LSL. Inhibition arrived sooner to the cell in the monotic condition compared to the dichotic (15.55 ms vs -0.29 ms, respectively). In other words, the monotic condition had leading

inhibition while the dichotic condition had lagging inhibition. The monotic condition displayed persistent inhibition because the duration of inhibition was longer than the NE tone, while the dichotic condition did not (Fig 4A and E). Spike count changes yielded different ISI values for T_1 and T_2 in the monotic vs the dichotic conditions, with the monotic condition having and a smaller T_1 and a larger T_2 (Fig 4B and F). Deviations in FSL and LSL values were different between conditions as well (Fig 4D and H).

Most cells tested with dichotic paired-tone stimulation exhibited ipsilateral inhibition at the BEF frequency (34 of 42, 80%); however, 20% did not. The second example cell that I am presenting represents a cell that did not have ipsilateral inhibition. This cell was a short-pass DTN that had measurable inhibition monotically (Fig. 5A). As before, in the monotic condition both the BD and NE tones were set to the cell's BEF (in this case 51 kHz and 37.5 dB SPL, respectively at +10 dB above threshold). Inhibition was detected with each criterion, and all gave different values for T_1 and T_2 (Fig. 5B, C, 2and D). The final estimates of T_1 and T_2 were -6 ms and 22 ms using a LSL and spike count criterion, respectively. This cell had 6.07 ms of leading inhibition observed in the monotic condition, with inhibition arriving to the cell 6.07 ms sooner than the cell's excitatory FSL (L_{First} = 7.98 ms and T_{start} = 1.91 ms). Persistent inhibition was also present in the monoitic condition, with inhibition lasting 6.08 ms longer than the duration of the NE tone.

In the dichotic condition, spike suppression was not visible in the dot raster display (Fig. 5E) and the effects of inhibition could not be detected using any of the three criteria. Spike counts did not decrease to < 50% of baseline (Fig. 5F), and there were no deviations in LSL or FSL (Fig. 5G and H).

I presented two examples of cells that were tested with monotic and dichotic paired-tone stimulation to clearly show two groups of cells that were present in my data – cells that had ipsilateral inhibition and cells that did not. When we compare the responses of the cell shown in Fig. 4 and Fig. 5, the main difference was apparent in the dichotic condition. No measurable inhibition was detected across spike count, LSL, or FSL at any of the 5 frequency points dichotically. The baseline spike count in the monotic condition (2.14 ± 0.59 spikes/stimulus) was similar to spike count sustained throughout paired-tone stimulation in the dichotic condition (2.26 ± 0.50 spikes/stimulus). Spiking which occurred between the T_1 and T_2 values monaurally decreased to 0.99 ± 0.41 spikes/stimulus. This demonstrates that some DTNs have contralateral inhibition without any measurable ipsilateral inhibition.

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

Cells within my sample had different response properties as frequency deviated from the BEF in the dichotic condition. I am going to present two examples of cells tested dichotically with paired-tone stimulation with both tones set to the cell's BEF and at equal amplitudes (+10 dB re BD threshold). The first example illustrates a neuron tested dichtically that showed measurable inhibition both when tested with the cell's BEF and at a non-BEF within the eBW (Fig. 6). The second example illustrates a neuron tested dichtically that showed measurable inhibition when tested with the cell's BEF but not when tested at a non-BEF outside the eBW (Fig. 7). The first example cell was a short-pass DTN that exhibited inhibition when tested dichotically at the cell's BEF (Fig.

6A). Inhibition lagged the cell's excitatory FSL by 0.82 ms in the BEF condition (T_{start} =17.49 ms, L_{First} = 16.61 ms). For the BEF condition, changes in the cell's spike count, FSL, and LSL (Fig. 6B, C, and D) each gave different T_1 and T_2 values. The final estimates of T_1 and T_2 were 2 ms and 40 ms and both were determined with changes in the evoked spike count.

In the non-BEF condition, the same cell was tested with the NE tone set to the $0.5H_{eBW}$ standardized frequency (47.5 kHz) within the cell's 50% eBW (Fig. 6E). The BD tone was set at the same parameters as (Fig. 6A), with both tones at equal amplitude. Inhibition was reduced at the $0.5H_{eBW}$ frequency but not abolished. Changes in the cell's spike count, FSL, and LSL (Fig. 6F, G, and H) each gave the same value for T_1 but gave different values for T_2. The final estimates of T_1 and T_2 were 4 ms and 22 ms and both were determined with changes in the evoked spike count.

In both the BEF and non-BEF conditions measurable inhibition was detected with at least one of the 3 criteria, which was done throughout the population of cells tested. Onset of inhibition, T_1, stayed relatively similar to the onset at the BEF condition while the offset of inhibition, T_2, decreased from 40 ms to 22 ms when departing from the BEF. Both conditions had lagging inhibition, as inhibition lagged the excitatory FSL by 0.82 ms in the BEF condition and increasing to 3.99 ms in the non-BEF condition. The BEF condition had persistent inhibition, with inhibition lasting 17.18 ms longer than the NE tone. The non-BEF condition did not have persistent inhibition, with inhibition ending 4.00 ms before the end of the NE tone.

The second example cell that I am presenting represents a cell that did not have measurable inhibition outside the cell's eBW. This cell was a band-pass DTN that had

measurable inhibition dichotically at the cell's BEF (Fig. 5A). Similar to the example given in Figure 6, the NE tone was presented to the ipsilateral ear and the BD tone was presented to the contralateral ear, and both tones were equal in amplitude (78.0 dB SPL or +10 dB re threshold). The contralateral BD tone was set to the BEF (28.0 kHz) and the ipsilateral NE was set to a non-BEF that was outside the eBW. Changes in the cell's spike count, FSL, and LSL (Fig. 7B, C, and D) each gave the same value for T_2 but gave different values for T_1. The final estimates of T_1 and T_2 were -1 ms and 43 ms and were determined with changes in LSL and spike count, respectively.

In this cell (Fig. 7), the non-BEF condition was tested with the NE tone at a frequency outside the cell's eBW ($1.5L_{eBW}$, 14.5 kHz). Spike suppression was not visible in the dot raster display (Fig. 7E). Inhibition was not detected using any of the 3 criteria. Spike counts did not decrease below 50% of baseline (Fig. 7F), and there were no deviations in the cell's FSL or LSL (Fig. 7G and H).

Baseline spike count in the BEF condition was 1.10 ± 0.84 spikes/stimulus over the first 10 positive ISIs and dropped to 0.05 ± 0.06 spikes/stimulus between the final T_1 and T_2 values chosen in the BEF condition. Spike counts in the non-BEF condition never deviated from baseline levels measured from the first 10 ISIs, maintaining 0.89 ± 0.86 spikes/stimulus through the entire paradigm. This demonstrates that inhibition was not observed when the NE tone frequency was outside the cell's eBW. Persistent inhibition was present in the dichotic BEF condition, with spike suppression maintained 11.39 ms longer than the duration of the NE tone. Inhibition was not present in the dichotic non-BEF condition.

A total of 42 DTNs were tested with dichotic paired-tone stimulation at the cell's BEF, 26 DTNs were tested in the monotic condition, and 33, 35, 32, and 31 DTNs were tested at the remaining 5 standardized frequency categories ($1.5L_{eBW}$, $0.5L_{eBW}$, $0.5H_{eBW}$, and $1.5H_{eBW}$, respectively). Spike suppression was always observed in the monotic condition (26/26; 100%) but 8 of 42 cells (20%) did not show evidence of measurable inhibition in the dichotic condition where both the BD and NE tone were set to the cell's BEF and were equal in amplitude. Indeed, only 7 of 29 cells (24%) had measurable inhibition at all 5 standardized frequencies.

Leading inhibition was observed in 24% (10/42 total) of cells tested with dichotic paired-tone stimulation at the BEF, compared to 50% (13/26) of cells tested with monotic paired-tone stimulation at the BEF. There were some cells that exhibited measurable inhibition in at least one of the 5 standardized frequencies ($1.5L_{eBW}$, $0.5L_{eBW}$, BEF, $0.5H_{eBW}$, or $1.5H_{eBW}$). In this group of cells, a greater percentage exhibited leading inhibition, greatest at the extreme frequencies, with proportions of cells at 5/16, 7/22, 10/34, 3/22, and 6/12, respectively. These proportions demonstrate that cells which have measurable inhibition outside the excitatory bandwidth ($1.5L_{eBW}$ and $1.5H_{eBW}$) were more likely to have leading inhibition. There was no difference in the proportion of cells with leading inhibition [$\chi^2(4) = 5.867$, $p = 0.209$, $n = 106$] across the 5 standardized NE tone frequencies in the binaural condition. When the monaural BEF condition was tested against the dichotic BEF condition, there was a difference in the duration of leading inhibition (Monotic = -1.48 ± 4.88 ms; Dichotic = 1.66 ± 6.29 ms; Mann–Whitney U = 313.00, $p = 0.038$). These findings show that onset of inhibition was very similar across

the 5 tested NE tones that were within 150% of the eBW of the cell but differed between monotic and dichotic conditions.

The amount of leading/lagging inhibition measured from the population of DTNs tested in the 5 standardized dichotic frequencies and in the monotic BEF condition was determined (Fig. 8). Leading inhibition was more likely in the monotic condition, with an average value of 2.19 ms – excitation arriving 2.19 ms after inhibition on average. Whereas 4 of the 5 frequency conditions on average displayed lagging inhibition (average lagging at $0.5L_{eBW}$ = - 0.63 $\pm$ 4.99 ms, BEF = - 1.48 $\pm$ 4.88 ms, $0.5H_{eBW}$ = - 4.55 $\pm$ 6.98 ms, and $1.5H_{eBW}$ = - 0.28 $\pm$ 5.40 ms) with only the $1.5L_{eBW}$ condition meeting criteria for leading inhibition ($1.5L_{eBW}$ = 0.45 $\pm$ 4.92ms).

Similarly, persistent inhibition (Fig. 8B) was generally observed in the monotic condition (15.97 ms). The dichotic conditions, on average, had spike suppression that lasted 9.58 ms shorter than the duration of the NE tone. When testing each cell at each of the 5 standardized frequencies in the dichotic condition, it was possible that inhibition could have been measured one frequency but not at another. When this occurred, the equation $D_{IHB} - D_{NE}$ used a value of 0 to represent D_{IHB} (duration of inhibition). Cells were only included in Figure 8 if inhibition was measured in at least 1 of the 5 frequency conditions. This essentially yielded a negative value of each cell's respective NE tone in conditions not exhibiting inhibition. For example, cell MU137.04 had a duration of inhibition of 13.7 ms. Since the NE tone was 40 ms long, this yielded a value of -26.3 in the calculation of relative duration of inhibition compared to the NE tone duration (13.7 – 40 = -26.3). However, there was no measurable inhibition at the $1.5L_{eBW}$ frequency category yielding a value of -40 (0 – 40 = -40). This frequency category was only

included in the dataset (Fig. 8A) because the BEF category had measurable inhibition at one of the criterion. There was a clear inverse parabolic pattern of persistent inhibition across the 5 conditions (averages at $1.5L_{eBW}$ = -12.58 $\pm$ 18.44 ms, $0.5L_{eBW}$ = - 7.51 $\pm$ 18.67 ms, BEF = - 3.34 $\pm$ 15.35 ms, $0.5H_{eBW}$ = - 9.73 $\pm$ 17.41 ms, and $1.5H_{eBW}$ = - 16.72 $\pm$ 16.50 ms). There was no difference in the proportion of cells with persistent inhibition [$\chi^2(4) = 1.726$, $p = 0.786$, $n = 106$] across the 5 standardized NE tone frequencies in the binaural condition. When the monaural BEF condition was tested against the dichotic BEF condition, there was a significant difference in the duration of persistent inhibition (Monotic = -2.07 ± 14.35 ms; Dichotic = 15.97 ± 16.35 ms; Mann–Whitney U = 163.00, $p << 0.05$).

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

I next describe the relation between leading/lagging inhibition and BD at each of the 5 standardized NE tone frequencies (Figure 9). There was a positive correlation between leading/lagging inhibition and BD for cells tested in the monotic condition ($R = 0.311$, $p = 0.002$), but there was no relationship between leading/lagging inhibition and BD at any of the 5 standardized dichotic frequency conditions (Table 1). Leading/lagging inhibition was longer in band-pass compared to short-pass DTNs in the monotic condition (band-pass = 5.83 ± 5.21 ms; short-pass = -1.39 ± 5.24 ms; Mann–Whitney U = 30.00, $p = 0.005$), but there was no relation between leading/lagging inhibition and duration filter class at any of the 5 standardized frequencies in the dichotic condition. One trend was evident: there was a tendency for band-pass cells to have greater leading inhibition compared to short-pass DTNs at all 5 standardized frequencies in the dichotic

condition ($1.5L_{eBW}$: BP = 1.97 ± 5.40, SP = -1.08 ± 4.18; $0.5L_{eBW}$: BP = 0.74 ± 5.89, SP = -1.99 ± 3.68; BEF: BP = 0.22 ± 4.31, SP = -3.00 ± 4.97; $0.5H_{eBW}$: BP = -2.08 ± 6.32, SP = -8.12 ± 6.62; and $1.5H_{eBW}$: BP = 1.35 ± 4.61, SP = -3.56 ± 5.99). There was a positive correlation between the amount of leading inhibition and FSL (Fig. 9B) in the monotic condition ($R^2 = 0.39$, $p = 0.001$) (Table 1), but there was no relationship between leading/lagging inhibition and FSL in the 5 dichotic conditions.

Comparing FRAs, BWs, Q factors, and BEFs and BIFs

By systematically varying the frequency of the BD tone to explore the range of excitatory frequencies, I was able to construct an eFRA and measure a BEF and eBW for each cell. I then used dichotic paired-tone stimulation and presented the ipsilateral NE tone at 5 standardized frequencies to construct iFRA and measure a BIF and iBW for each cell. When inhibition was still present at the $1.5L_{eBW}$ and $1.5H_{eBW}$ conditions, I continued to test the cell with NE tone frequencies above and below these values until inhibition was abolished or until the cell was lost.

Of the 25 cells where an iBW could be calculated, 13 (52.0%) had iBWs that were narrower than the cell's eBW and 12 had an iBW that was broader than the cell's eBW. For example, the eFRA measured from a short-pass DTN with a BEF of 43.0 kHz and an eBW of 3 kHz (ranging from 41.0 to 44.0 kHz, or -0.0687 to + 0.0332 octaves re BEF) was recorded (Fig 10A). The excitatory $Q_{10\ dB}$ value of the cell (i.e. Q_{10dB} = BEF/eBW) was 14.3, indicating relatively sharp excitatory frequency tuning. Some cells did not have a BEF and BIF that exactly matched. The cell showed in Fig. 10B had a BIF of 38.0 kHz and an iBW of 13.7 kHz (ranging from 32.2 to 45.9 kHz, or -0.418 to +0.094

octaves re BEF). This neuron was tested with 12 NE tone frequencies during dichotic paired-tone stimulation ranging from $9.5L_{eBW}$ to $4.5H_{eBW}$. The cell's iBW was ~4.5x broader than its eBW. The inhibitory Q value (Q = BIF/iBW) measured at +10 dB (re BD, BEF threshold) was 3.13, revealing very broadly tuned neural inhibition. The low- and high-frequency slopes of its normalized iFRA were $Slope_{Low}$ = 2.332 and $Slope_{High}$ = -4.389. The entire eBW was overlapped by the iBW, with most of the iBW extending to lower frequencies (compare width of grey boxes in Fig. 10A and B).

The excitatory and inhibitory eFRAs of a short-pass DTN where the BEF matched the BIF to within 0.05 octaves of the BEF was recorded (Fig 10C and D). The eFRA with a BEF of 43.0 kHz and an eBW of 5 kHz (ranging from 41.0 to 46.0 kHz, or -0.069 to 0.097 octaves re BEF) was measured (Fig. 10C). The excitatory $Q_{10\ dB}$ value was 8.6, indicating relatively sharp excitatory tuning. The same cell had a BIF of 42.0 kHz and a iBW of 17.06 kHz (ranging from 30.5 to 47.6 kHz; or -0.472 to 0.144 octaves re BIF, Fig 10D). A total of 13 NE tone frequency conditions were recorded from this cell binaurally, ranging from $10L_{eBW}$ on the lower frequency area to $3.0H_{eBW}$ on the higher frequency area. The iBW was ~3.4x broader than the eBW. The inhibitory Q value (Q = BIF/iBW) measured at +10 dB (re BD, BEF threshold) was 2.46, revealing broadly tuned neural inhibition. The low- and high-frequency slopes of the normalized iFRA were $Slope_{Low}$ = 0.845 and $Slope_{High}$ = -3.304. As in the previous cell, at higher frequencies, inhibitory tuning was sharper at the frequencies that I tested. The entire eBW was overlapped by the iBW, with the majority of the iBW extending to lower frequencies (compare width of grey boxes in Fig. 10C and D). These data provide evidence that the strength of inhibition in this cell was maintained over a broad range of NE tone frequencies.

Across the population of cells tested, the average difference between the BIF and BEF was less than 0.05 octaves and was significantly different than 0 (mean = 0.0465 $\pm$ 0.037 $p > 0.05$, $n = 25$). This demonstrates that the contralateral BEF and ipsilateral BIF were closely matched in frequency. There were 6 cells (24%) in which the BIF and BEF matched exactly, 7 cells (28%) that differed between 0 and $< \pm$ 0.05 octaves, 10 cells (40%) that were $> \pm$ 0.05 and $< \pm$ 0.20 octaves from BEF, and only 2 cells (8%) that differed by $> \pm$ 0.20 octaves (Fig. 11). Therefore, the same range of frequencies that elicit maximal excitation are the frequencies that elicit maximal inhibition ipsilaterally.

Sharpness of excitatory ($Q_{10\ dB}$) and inhibitory (inhib Q) tuning was compared between contralateral and ipsilateral inputs measured with monotic and dichotic paired-stimulation. No difference was observed between excitatory (8.50 $\pm$ 6.99) and inhibitory (8.03 $\pm$ 10.69) Q factors (Fig. 12; $F = 0.129$, $p = 0.856$, $n = 25$).

I also compared the steepness of the low-frequency tuning slope ($Slope_{Low}$) to the steepness of the high-frequency slope ($|Slope_{High}|$) for each iFRA. This was done to examine the symmetry of the ipsilaterally-evoked inhibitory tuning. There was no difference between the $Slope_{Low}$ values (mean = 8.94 $\pm$ 14.26) and the $|Slope_{High}|$ values (mean = 9.60 $\pm$ 12.74) within my population of DTNs ($F = 0.017$, $p = 0.898$, $n = 25$). There were 11 cells (44%) that had a $Slope_{Low}$ that was steeper than $|Slope_{High}|$; the remaining 14 cells (56%) had a $|Slope_{High}|$ value that was steeper than the corresponding $Slope_{Low}$ (Fig. 13).

Ipsilateral cell classifications - EE, EI, or E0

A total of 22 cells were classified as either EE (contralateral ear excitatory, ipsilateral ear excitatory), EI (contralateral ear excitatory, ipsilateral ear inhibitory), or E0 (contralateral ear excitatory, no ipsilateral ear input). There were 12 cells (55%) that were EI, 5 (22.5%) that were EE, and 5 (22.5%) that were E0. There were 2 cells that met exclusionary criteria and were removed because they were responded at an inter-level difference that was > 30dB and, thus, I could not rule out acoustic cross talk as a confounding factor. One cell was unique in its firing pattern, meeting inclusion criteria for the EE category because the ipsilateral excitation threshold was 30 dB lower than the contralateral excitatory threshold, as well as the EI category (spike suppression to <50% when varying ipsilateral amplitude, holding contra at + 10 dB re threshold). (Wenstrup et al., 1988; Shen et al., 1997)

Discussion

Monaural and binaural inhibitory inputs to DTNs

Several studies have found that DTN neural circuits are created exclusively with monaural excitatory and inhibitory inputs offset in time (Casseday et al., 1994, 2000; Covey et al., 1996; Fuzessery and Hall, 1999; Faure et al., 2003; Leary et al., 2008; Aubie et al., 2009; Valdizón-Rodríguez and Faure, 2017). We do know that a subset of DTNs have both excitatory and inhibitory ipsilateral inputs (Sayegh et al., 2014a, 2014b). It is theoretically possible to have duration tuning without inhibition in some proposed models such as the coincidence detection model (Aubie et al., 2009; Sayegh et al., 2011), but contralaterally-evoked inhibition is clearly present DTNs. The presence of inhibition

has been supported by prior research where pharmacological antagonists of the inhibitory neurotransmitters GABA and glycine have been applied to DTNs in the midbrain, leading to the broadening or complete abolishment of duration tuning (Casseday et al., 1994, 2000; Fuzessery and Hall, 1999). The role of ipsilateral inhibition in duration tuning is still unknown.

In the current study, a BD tone was used as a probe whose onset was varied relative to the onset of an NE tone which reliably suppressed BD tone-evoked spiking. I had found that all cells tested with pair-tone stimulation in the monaural condition with equal amplitude and frequency consistently had NE tone-evoked inhibition (100% of cells). However, 20% of cells did not display inhibition binaurally when the NE tone was presented ipsilaterally and the BD tone was presented contralaterally. This indicates that some DTNs can be formed completely independent of ipsilateral inputs, but that ipsilateral inhibitory inputs are likely utilized to some extent to modulate monaural inputs. The proportion of cells that had ipsilateral inhibition in my study was greater than previous studies (Sayegh et al., 2014a; Mastroieni 2017), but may have been due to the greater number of band-pass DTNs in my tested population (45% vs 35%). In my study, and in previous studies, band-pass DTNs have been found to have longer durations of leading inhibition (Faure et al., 2003; Sayegh et al., 2014b; Valdizón-Rodríguez and Faure, 2017).

In the monotic condition, most cells had a latency of inhibition that was shorter than the excitatory FSL (i.e. leading inhibition; Faure et al., 2003; Sayegh et al., 2014; Valdizón-Rodríguez and Faure, 2017). During monotic testing most cells also had inhibition that lasted as long or longer than the duration of the NE tone evoking the

inhibition (i.e. persistent inhibition). These monotic observations indicate that DTNs receive an onset-evoked inhibitory input that arrives before an onset-evoked excitatory input, and that the inhibition is sustained beyond the duration of the stimulus (Casseday et al., 1994, 2000).

Of the cells that displayed ipsilaterally-evoked inhibition during dichotic paired-tone stimulation, most had lagging inhibition (i.e. the onset of inhibition was slower than the onset of excitation) that did not last as long as the NE tone evoking the inhibition (Figure 8). Fewer DTNs showed evidence of having ipsilateral inhibitory inputs, and when inhibition was present, it was shorter in duration and arrived at a longer latency. This provides evidence that the inhibition necessary for duration tuning is primarily contributed monaurally (Sayegh et al., 2014).

In summary, ipsilateral inhibition is clearly shorter, weaker, and arrives later than contralateral inhibition that creates DTNs. In previous studies, the proportion of DTNs which did not show evidence of ipsilateral inhibition (~50%) began showing inhibition when the amplitude of the NE tone was increased (Sayegh et al., 2014). At +20 dB above threshold, a majority of cells showed evidence of ipsilateral inhibition. This suggests that ipsilateral inputs are present but possibly have higher thresholds than contralateral inhibition. All of this indicates that duration selectivity is primarily formed through monaural (contralateral) auditory pathways, with ipsilateral inputs, when present, acting to modulate contralaterally-evoked responses.

Varying the NE tone frequency in dichotic paired-tone stimulation

Response evoked by NE tones of different frequencies showed inhibition that was quite different from the monaural case. Previous studies testing the latency of inhibition monaurally at the same 5 standardized frequencies found that latency of inhibition remained relatively constant, while the offset and/or time course of inhibition decreased systematically as the NE tone frequency deviated from the BEF (Valdizón-Rodríguez and Faure, 2017). In my study, the onset of inhibition changed when comparing the monotic BEF condition to the dichotic BEF condition. This change represents a switch from one classification to another (leading inhibition to lagging inhibition). However, after the shift in inhibitory latency from monotic to dichotic conditions, the dichotic inhibitory latency remained fairly constant at each of the 5 standardized frequencies. Thus, as with contralaterally-evoked inhibition, the onset of ipsilateral inhibition was relatively frequency independent but was delayed relative to contralateral inhibition in the same neuron.

A previous monural paired-tone study that manipulated the NE tone frequency found that the duration of inhibition systematically decreased as the NE tone frequency deviated from the BEF (Valdizón-Rodríguez and Faure, 2017). This was also the case in my study for dichotic paired-tone stimulation, with a significant difference in the relative duration of inhibition (re NE tone) between the monotic and dichotic conditions at the BEF, and with a decrease is the number of cells exhibiting inhibition as the NE tone frequency deviated from the BEF (Fig. 8). When the NE tone was set to the BEF, 80% of cells showed evidence of ipsilateral inhibition but only 24% of cells showed ipsilateral inhibition at all 5 standardized frequencies (10/42 cells). This demonstrates that the offset

of ipsilateral inhibition was frequency dependent, a finding similar to the results of contralateral inhibition measured with monaural paired-tone stimulation (Valdizón-Rodríguez and Faure, 2017). These findings indicate that there may be at least 2 sources of inhibititory inputs to DTNs: an onset-evoked, frequency independent, transient inhibitory input, and an onset-evoked, sustained, frequency dependent, inhibitory input. Previous neuropharmacalogical studies using GABA and glycine (inhibitory neurotransmitters) antagonists have found that there was a greater lengthening of the excitatory response period in cells when GABAergic inhibition was blocked than when glycinergic inhibition was blocked (Casseday et al., 2000). This finding suggests that GABAergic inhibition might underlie the sustained inhibition, while glycinergic inhibition may be responsible for the fast onset, transient inhibition acting on DTNs.

Leading inhibition is responsible for lengthening FSLs with increases in sound pressure level (Covey et al., 1996). This is known as paradoxical latency shift, named 'paradoxical' because it is opposite the trend seen in most auditory neurons where the FSL decreases with increasing stimulus energy. Paradoxical shift is caused by an increase in the duration of leading inhibition coinciding with increasing amplitude which appeared to cancel out the initial excitatory FSL found in a small subset of DTNs (Covey et al., 1996; Fremouw et al., 2005). Sayegh et al. (2014) reported that when the amplitude of the ipsilateral NE tone was increased to + 20 dB re the contralateral BD tone in dichotic testing, most cells that did not show spike suppression when the two tones were equal in amplitude now showed evidence of ipsilateral inhibition. It is likely that due to the level increase ipsilaterally, a greater inhibitory response from the ipsilateral ear was recruited. In any case, leading inhibition in the monaural circuit has been found to determine, or

play a significant role in, the duration filter class, BD, and FSL, meaning its importance for shaping duration tuning is clear (Casseday et al., 1994, 2000, Aubie et al., 2009, 2012).

In the current study, cells tested in the monotic condition showed a relationship between leading inhibition and BD, FSL, and duration filter class. This was as reported in previous studies (Valdizón-Rodríguez and Faure, 2017). In my study, there was no correlation between the onset of inhibition and FSL or duration filter class across the 5 standardized NE tone frequencies. Similarly, there was no correlation between leading inhibition and BD across the 5 standardized NE tone frequencies. Valdizón-Rodríguez & Faure (2017) found that the correlation between leading inhibition and BD, FSL, and duration filter class was consistent across the 5 standardized NE tone frequencies monaurally with exception of the $1.5H_{eBW}$ condition. They also found that the duration of leading inhibition increased in DTNs tuned to longer BDs and FSLs.

My sample had a larger proportion of band-pass DTNs than Sayegh et al. (2014). The number of cells which had measurable inhibition decreased the further the NE tone frequency moved away from the BEF, but the proportion of band-pass cells in those groups increased. This implies that band-pass cells are more likely to show leading inhibition at a greater range of frequencies in the monotic and dichotic conditions (Table 2). This could be a reason why I had a greater proportion of cells with ipsilateral inhibition compared to previous studies (Sayegh et al., 2014b; Valdizón-Rodríguez and Faure, 2017).

Comparing the excitatory and inhibitory bandwidths

I found that about half (48%) of cells with ipsilateral inhibition had iBWs that were broader than their contralateral eBWs. In the cases where iBWs were broader than eBWs, most iBWs overlapped the eBW entirely and extended further toward lower frequencies (Figure 10). For inhibition to shape excitatory inputs, it would require inhibitory inputs that either matched the eFRA or covered a broader range of frequencies. This is true monaurally, where Valdizón-Rodríguez & Faure (2017) identified that the iBWs of DTNs were broader than the corresponding eBWs. They also found that onset of contralateral inhibition was frequency independent and suggested that this feature may help to maintain duration tuning at non-BEF frequencies. However, this same finding was not observed in dichotic testing, with half of DTNs having ipsilateral iBWs that were narrower than their contralateral eBWs. This provides evidence that binaural inputs likely play modulatory roles and do not provide the primary inhibition that forms DTN circuits ipsilaterally. If ipsilateral-evoked inhibition provided the inhibition necessary to form a duration-tuned circuit, then the inhibitory range should match or overlap the excitatory range. This matched range would maintain duration selectivity across the eFRA by allowing the inhibition to suppress spiking long enough to coincide with offset-evoked excitatory inputs. This is the basis of the coincidence detection model for DTNs (Aubie et al., 2009, 2012).

I found that the slopes of the low-frequency ($Slope_{Low}$) iFRA did not differ compared to the absolute value of the high-frequency slopes ($|Slope_{High}|$). In DTNs tested with monaural paired-tone stimulation, the magnitude of the high-frequency slopes were greater than low-frequency slopes (Valdizón-Rodríguez and Faure, 2017), meaning

these cells are asymetrically tuned. This indicates that monoaural (contralateral) inhibition was strongest at and below the BIF. Cells in my data that showed the same monaural trend $Slope_{Low} < |Slope_{High}|$, had slope magnitudes that were an average of 4x larger than the low frequency. This is less compared to an average of 2x larger slope magnitudes in the $Slope_{Low} > |Slope_{High}|$ group. This finding demonstrates a clear difference between binaural and monaural paired-tone stimulation iBW slopes but also shows that some cells display similar inhibitory patterns as monaural circuits. It is possible that the asymmetry in the flanks of the inhibitory tuning is derived from cells innervating the IC from columnar cells of the ventral nucleus of the lateral meniscus ($VNLL_c$). These $VNLL_c$ neurons are broadly tuned to frequency, matching the broad iBW observed monaurally, and have high frequency tuning curve flanks that are steeper than their low frequency tuning flanks (Covey and Casseday, 1991). Cells from the $VNLL_c$ respond with a single, constant latency spike (Covey and Casseday, 1991), and are known to have glycinergic projections (Vater et al., 1997), possibly explaining the initial onset-evoked inhibition. Some cells found in both the intermediate and ventral nucleus of the lateral lemniscus (INLL and VNLL) respond tonically with constant spike discharges and a large number of which stain for GABA, glycine, or both (Vater et al., 1997). Constant activation from the INLL and $VNLL_c$ neurons could possibly be supplying the GABA or glycine providing the inhibitory synaptic inputs to DTNs necessary to explain their onset-evoked, sustained inhibition.

Role of ipsilateral and contralateral inhibition

The inhibition evoked contralaterally in DTNs differed from that evoked ipsilaterally in three main respects: 1) inhibition lead excitation in the monotic condition, but lagged excitation in the dichotic condition; 2) duration of inhibition persisted as long or longer than the NE tone in the monotic condition, but lasted shorter than the duration of the evoking tone in the dichotic condition; 3) iBWs were broadly tuned and completely overlapped the eBW in the monotic condition, while there was a mixture of broad and narrow iBWs in the dichotic condition. There were several similarities between the contralateral and ipsilateral-evoked inhibitions in DTNs: 1) BIFs and BEFs were matched in both conditions; 2) the duration of inhibition decreasing as the stimulus frequency departed from the BEF; 3) the onset of inhibition was frequency independent when measured monotically and dichotically, meaning that the latency of the evoked inhibition did not change across different frequencies. All monaural evidence is taken from Valdizon-Rodriguez and Faure (2017).

Unpacking the similarities and differences of the inhibition evoked in monotic and dichotic listening can help us understand the neural circuits that create and modify temporal perception. Because ipsilaterally-evoked inhibition was more likely to contribute lagging inhibition to DTNs, and because the relative onset of inhibition plays a significant role in temporal tuning (Covey et al., 1996), it is possible that binaural inputs are utilized to provide inhibition within the same range as the higher frequency portion of the eFRA. Because ipsilateral iBWs could be broader or narrower than contralateral eBWs, this means that some DTNs receive more or less ipsilateral inhibition. Of cells with greater iBWs than eBWs, 64% were band-pass DTNs, a trend that might indicate a

greater ipsilateral influence of ipsilateral inhibition than in short-pass DTNs. Onset of inhibition was seen to be frequency independent in both cases, possibly indicating inputs originating from neurons with similar properties or locations. Some possible candidate auditory sources could be the $VNLL_c$ and INLL monaurally (Casseday et al., 2000; Valdizón-Rodríguez and Faure, 2017) and the dorsal nucleus of the lateral lemniscus (DNLL) binaurally due to its known bilateral projections (Covey and Casseday, 1986, 1991). The transient, offset-evoked excitation to DTNs could come from the medial superior olive which has projections to the central nucleus of the IC (Covey and Casseday, 1991; Grothe and Casseday, 1998). Additionally, if binaural ipsilateral innervations originate from the DNLL, it would further explain the absence of leading inhibition during dichotic but not monotic paired-tone stimulation. Projections from the DNLL are GABAergic (Shneiderman et al., 1993), while $VNLL_c$ neurons are both glycinergic and GABAergic (Casseday et al., 2000). A lack of glycinergic inputs, a possible candidate for the initial onset-evoked inhibition acting on DTNs (Casseday et al., 2000), may help to explain why lagging inhibition was observed ipsilaterally. Because glycine is possibly the major contributor of the initial transient inhibition observed in DTNs monotically, and the dichotic pathway is only affected by GABAergic innervations, this is possibly causing the sustained inhibitory response with no initial transient inhibition.

My results show that DTNs likely receive at least two types of inhibitory inputs. The first type is monaural in nature, was observed in all cells, and was evoked by stimulation of the contralateral ear. This inhibition alone can create the temporally selective responses observed in DTNs. The second type of inhibition was only seen in a

subset of DTNs and was evoked from stimulation of the ipsilateral ear. Future studies should determine how binaural inputs possibly affect iBWs in the monaural circuit. Paired-tone stimulation can be presented contralaterally while simultaneously stimulating the ipsilateral ear with the same NE tone. This could lead to a better understanding of how neural circuits are integrated, what modulatory role ipsilateral inhibition plays, and why binaural properties are different in some ways but similar in others.

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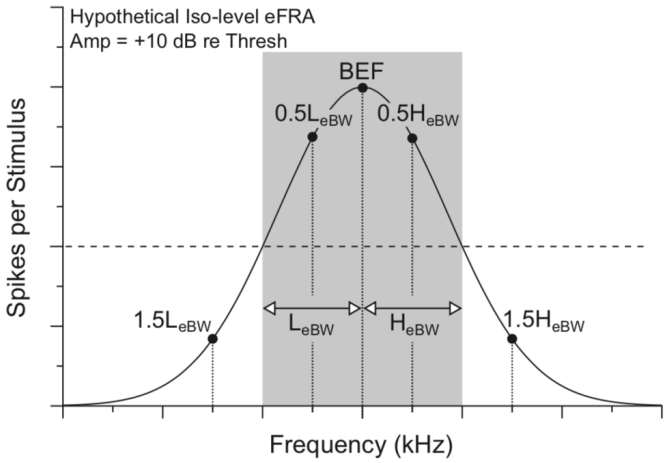

Fig. 1: Determining the 5 standardized NE tone frequencies. Duration-selective neurons were tested with paired-tone stimulation using 5 NE tone frequencies that were standardized relative to the eBW (gray box) of each cell. The figure illustrates a hypothetical isolevel eFRA of a DTN, obtained by recording 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 was used to determine the 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.

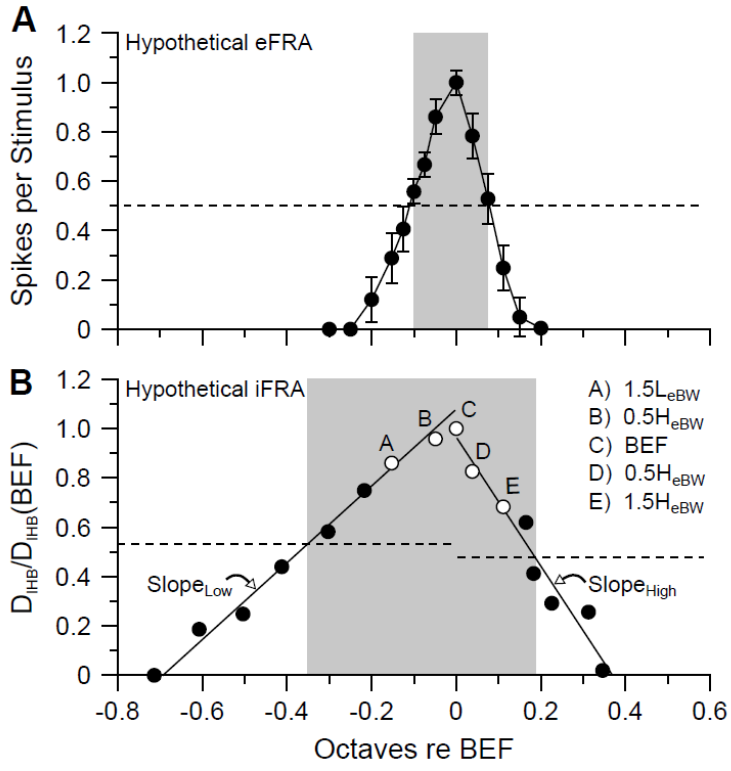

Fig 2: Determining the excitatory and inhibitory FRAs, BEF, eBW, BIF, and iBW. **A**: Hypothetical isolevel eFRA of a DTN showing the mean ± SD spikes per stimulus in response to single BD tones randomly varied in frequency and presented at +10 dB re threshold (filled circles). The eBW (gray box) was defined as the span between the lowest and highest frequencies at the 50% spike count re BEF (horizontal dashed line). See Fig. 1 for additional details. **B**: Hypothetical isolevel iFRA in 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. 1 (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 used to measure 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).

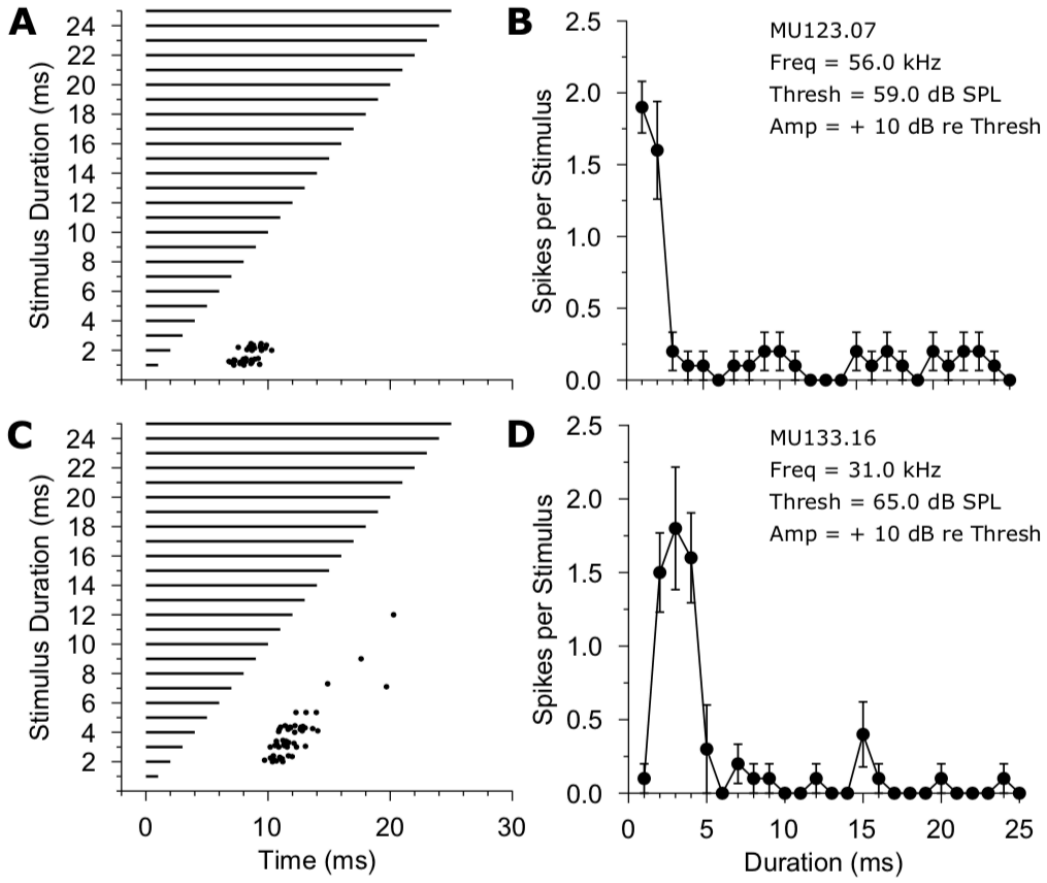

Fig. 3: Raster displays and duration tuning curves of DTNs recorded from the IC of the big brown bat. **A** and **C**: Short-pass DTN and a Band-pass DTN duration raster plot displaying the timing of evoked action potentials in response to suprathreshold best excitatory frequency (BEF) tones that were randomly varied in duration. **B** and **D**: Mean ± standard error (SE) spikes per stimulus as a function of stimulus duration for suprathreshold BEF tones. n = 10 trials per stimulus.

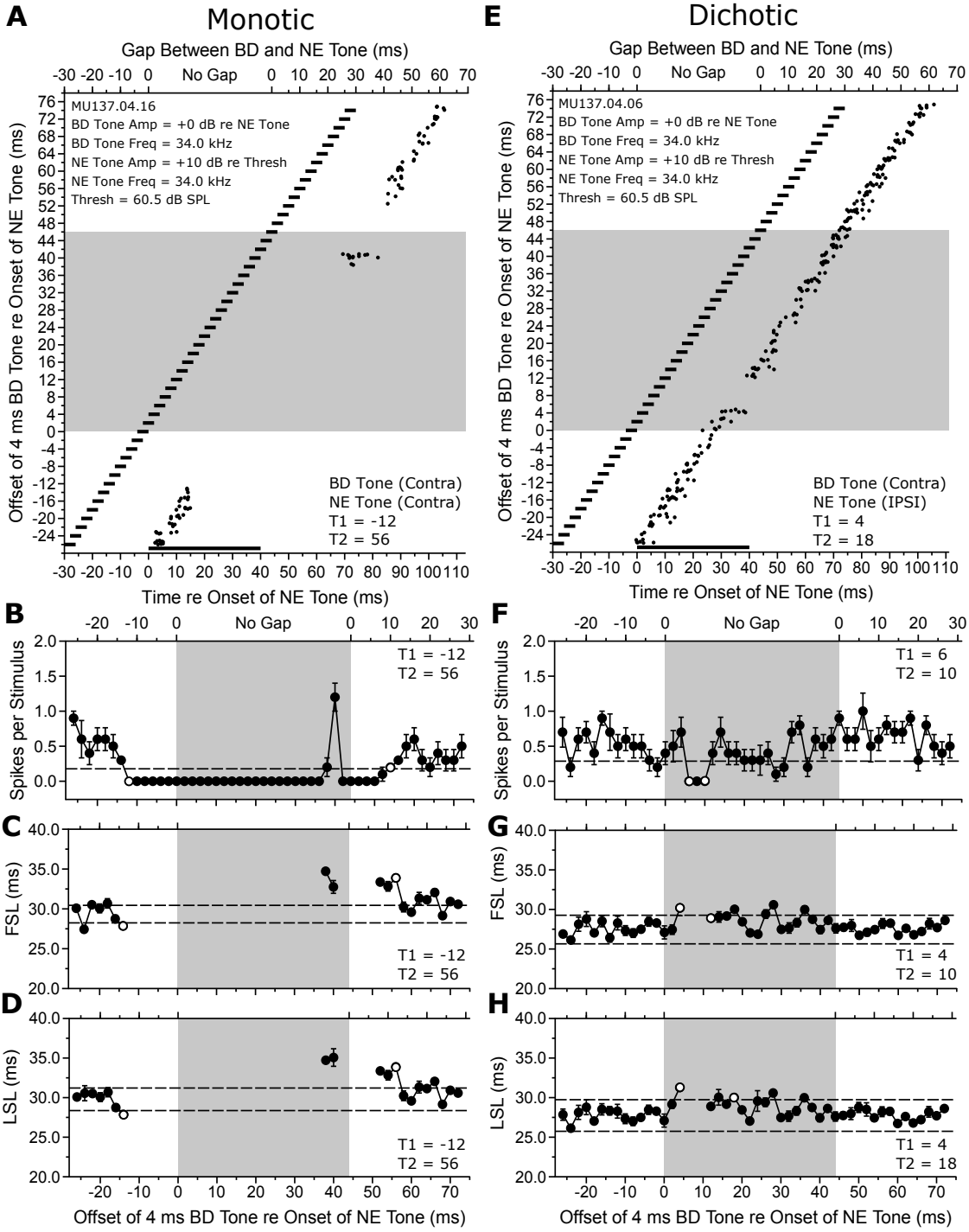
A
Monotic
Gap Between BD and NE Tone (ms)
MU137.04.16
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 34.0 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 34.0 kHz
Thresh = 60.5 dB SPL
BD Tone (Contra)
NE Tone (Contra)
T1 = -12
T2 = 56
Offset of 4 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
E
Dichotic
MU137.04.06
BD Tone (Contra)
NE Tone (IPSI)
T1 = 4
T2 = 18
B
Spikes per Stimulus
F
T1 = 6
T2 = 10
C
FSL (ms)
G
D
LSL (ms)
H
Offset of 4 ms BD Tone re Onset of NE Tone (ms)

Fig. 4: Inhibition evoked by monotic and dichotic paired-tone stimulation at the BEF. Dot raster displays illustrating responses from a band-pass DTN with the NE tone presented to the contralateral ear (34.0 kHz; left) and then presented to the ipsilateral ear (right). **A**: Strong spike suppression was observed when the 4-ms BD tone and the 40-ms NE tone were sufficiently close in time. **B**: Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones. The first ISI in which spike count dropped to < 50% of baseline was T_1= - 12 ms (leftmost open circle). The last ISI, starting from T_1, in which the spike count remained at < 50% of baseline was T_2 = 56 ms (rightmost open circle). **C**: Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The first ISI in which the FSL remained deviated by $\geq$ 1 SD from baseline was T_1 = - 12 ms (leftmost open circle), and the last ISI in which the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 56 ms (rightmost open circle). **D**: Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The first ISI in which the LSL deviated by 1 SD from baseline was T_1 = - 12 ms (leftmost open circle), and the second ISI in which the FSL deviated by 1 SD from baseline was T_2 = 56 ms (rightmost open circle). In the monotic condition, the final values of T_1 (- 12 ms) and T_2 (56 ms) were concurrent with all 3 criteria. The onset of the NE tone-evoked inhibition led the excitatory FSL by 15.55 ms, and the inhibition persisted 27.55 ms longer than the NE tone. **E**: Dot raster display illustrating responses from the same DTN when the BD tone was presented contralaterally and the NE tone was presented ipsilaterally. **F**: Inhibition evoked by the NE tone also led to a reduction in the cell's spike count, although the effective duration of spike suppression was shorter in duration and longer in latency. Deviations were observed in both the (**G**) FSL and (**H**) LSL. The final value of T_1 (4 ms) was determined with either a FSL or LSL criterion, and the final value of T_2 (18 ms) was determined with the FSL criterion. In the dichotic condition, the latency of inhibition lagged the excitatory FSL by 0.29 ms, and the duration of inhibition was 13.71 ms shorter than the NE tone. n = 10 trials per stimulus.

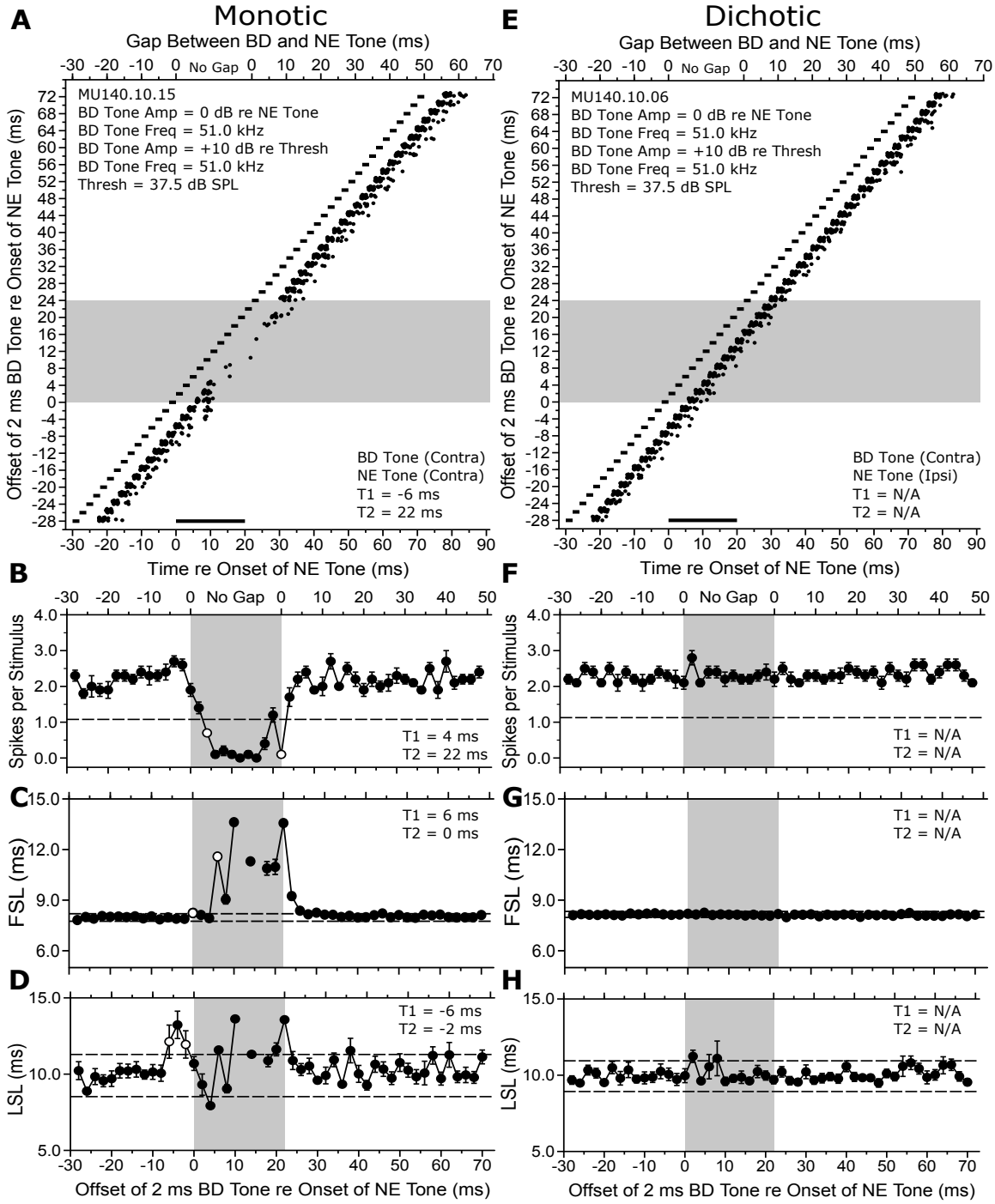
A
Monotic
Gap Between BD and NE Tone (ms)
MU140.10.15
BD Tone Amp = 0 dB re NE Tone
BD Tone Freq = 51.0 kHz
BD Tone Amp = +10 dB re Thresh
BD Tone Freq = 51.0 kHz
Thresh = 37.5 dB SPL
BD Tone (Contra)
NE Tone (Contra)
T1 = -6 ms
T2 = 22 ms
Offset of 2 ms BD Tone re Onset of NE Tone (ms)
E
Dichotic
Gap Between BD and NE Tone (ms)
MU140.10.06
BD Tone Amp = 0 dB re NE Tone
BD Tone Freq = 51.0 kHz
BD Tone Amp = +10 dB re Thresh
BD Tone Freq = 51.0 kHz
Thresh = 37.5 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = N/A
T2 = N/A
Offset of 2 ms BD Tone re Onset of NE Tone (ms)
B
Time re Onset of NE Tone (ms)
Spikes per Stimulus
T1 = 4 ms
T2 = 22 ms
F
Time re Onset of NE Tone (ms)
Spikes per Stimulus
T1 = N/A
T2 = N/A
C
FSL (ms)
T1 = 6 ms
T2 = 0 ms
G
FSL (ms)
T1 = N/A
T2 = N/A
D
LSL (ms)
T1 = -6 ms
T2 = -2 ms
Offset of 2 ms BD Tone re Onset of NE Tone (ms)
H
LSL (ms)
T1 = N/A
T2 = N/A
Offset of 2 ms BD Tone re Onset of NE Tone (ms)

Fig. 5: Inhibition evoked by monotic but not dichotic paired-tone stimulation at the BEF. Dot raster displays of responses from a short-pass DTN with the NE tone first presented to the contralateral ear (monotic) and then to the ipsilateral ear (dichotic). **A**: Spike suppression was observed when the 2-ms BD tone and the 20-ms NE tone were sufficiently close in time. **B**: Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones. The first ISI in which spike count dropped to < 50% of baseline was T_1 = 4 ms (leftmost open circle). The last ISI, starting from T_1, in which the spike count remained at < 50% of baseline was T_2 = 22 ms (rightmost open circle). **C**: Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The first ISI in which the FSL deviated by $\pm$ 1 SD from baseline was T_1 = 0 ms (leftmost open circle), and the last ISI in which the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 6 ms (rightmost open circle). **D**: Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The first ISI in which the LSL deviated by $\geq$ 1 SD from baseline was T_1 = - 6 ms (leftmost open circle), and the last ISI in which the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = - 2 ms (rightmost open circle). In the monotic condition, the final values of T_1 (- 6 ms) and T_2 (22 ms) were determined using spike count and LSL criteria, respectively. In the monotic condition, the onset of the NE tone-evoked inhibition led the excitatory FSL by 6.07 ms, and inhibition persisted 6.08 ms longer than the NE tone. **E**: Dot raster display illustrating responses from the same DTN when the BD tone was presented contralaterally and the NE tone was presented ipsilaterally. No spike suppression was observed in any of the criteria tested; no change in spike count (**F)** and no deviations in either (**G**) FSL or (**H**) LSL. n = 10 trials per stimulus.

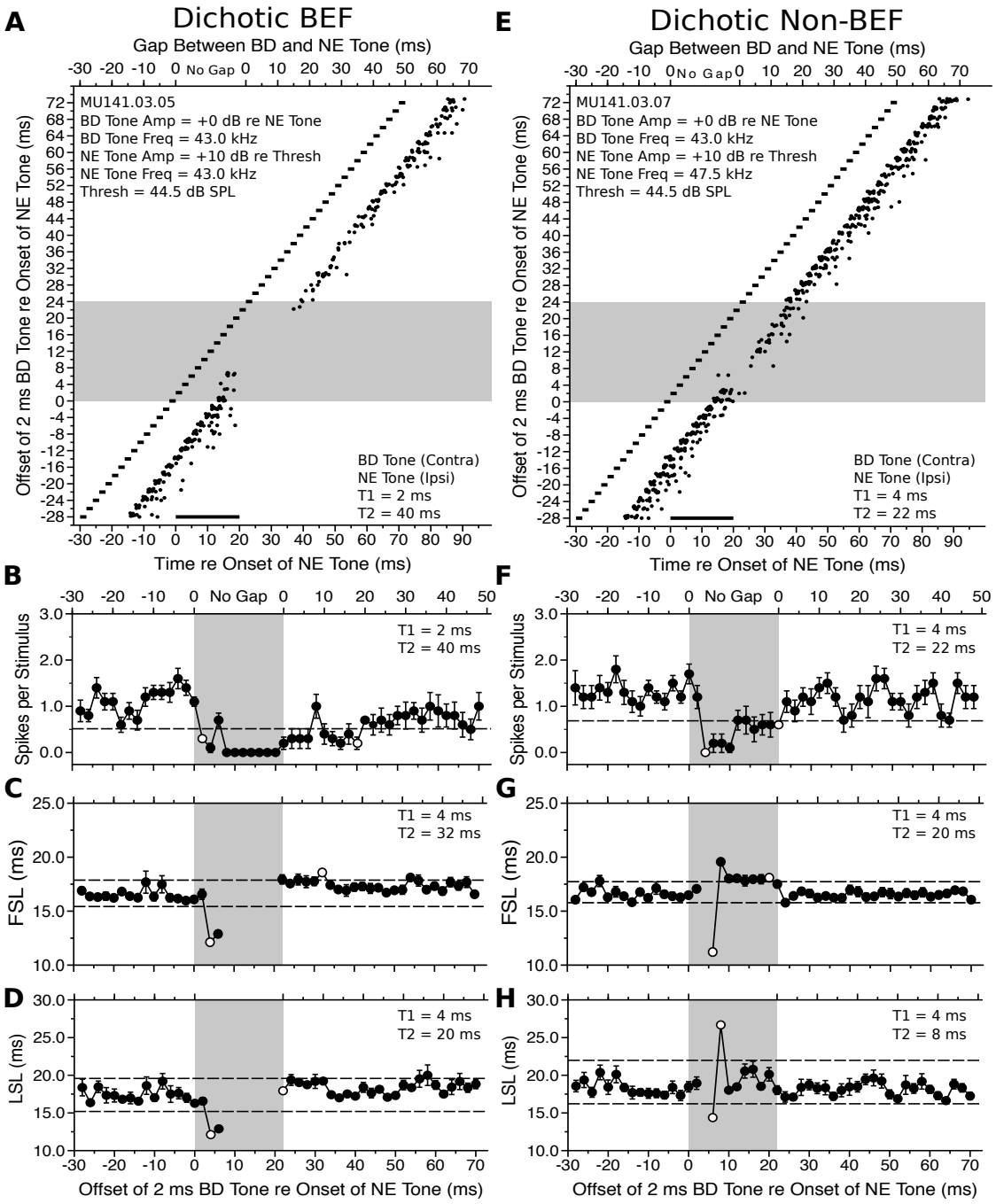
A
Dichotic BEF
Gap Between BD and NE Tone (ms)
MU141.03.05
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 43.0 kHz
NE Tone Amp = +10 dB re Thresh
NE Tone Freq = 43.0 kHz
Thresh = 44.5 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = 2 ms
T2 = 40 ms
Offset of 2 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
E
Dichotic Non-BEF
MU141.03.07
NE Tone Freq = 47.5 kHz
T1 = 4 ms
T2 = 22 ms
B
F
Spikes per Stimulus
T1 = 2 ms
T2 = 40 ms
T1 = 4 ms
T2 = 22 ms
C
G
FSL (ms)
T1 = 4 ms
T2 = 32 ms
T1 = 4 ms
T2 = 20 ms
D
H
LSL (ms)
T1 = 4 ms
T2 = 20 ms
T1 = 4 ms
T2 = 8 ms
No Gap
Offset of 2 ms BD Tone re Onset of NE Tone (ms)

Fig. 6: Dichotic paired-tone stimulation at the BEF and at a non-BEF within the 50% eBW. Dot raster displays of responses from a short-pass DTN with the BD tone presented to the contralateral ear and the NE tone presented to the ipsilateral ear at the cell's BEF (43.0 kHz; left). The non-BEF was within the eBW (47.5 kHz; right). **A**: When the BD and NE tones were matched at the cell's BEF, spike suppression was observed when the 2-ms BD tone and the 20-ms NE tone were sufficiently close in time. **B**: Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones. The first ISI in which the spike count dropped to < 50% of baseline was T_1 = 2 ms (leftmost open circle). The last ISI, starting from T_1, in which the spike count remained < 50% of baseline was T_2 = 40 ms (rightmost open circle). **C**: Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The first ISI when the FSL deviated by $\geq$ 1 SD from baseline was T_1 = 4 ms (leftmost open circle), and the second ISI in which the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 32 ms (rightmost open circle). **D**: Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The first ISI in which the LSL deviated by $\geq$ 1 SD from baseline was T_1 = 4 ms (leftmost open circle), and the last ISI when the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 20 ms (rightmost open circle). In the BEF condition, the final values of T_1 (2 ms) and T_2 (40 ms) were both determined with a spike count criterion. In the BEF condition, the onset of the NE tone-evoked inhibition lagged the excitatory FSL by 0.82 ms, and inhibition did not persist longer than the NE tone. **E**: Dot raster display of responses from the same DTN when the BD was at the BEF and the NE tone was at $0.5H_{eBW}$. **F**: Inhibition evoked by the NE tone again caused a reduction in the cell's spike count and deviations in the (**G**) FSL and (**H**) LSL, although the effective duration of the altered response was much shorter. The final values of T_1 (4 ms) and T_2 (22 ms) were determined with a spike count criterion. In the non-BEF condition, the latency of inhibition lagged the excitatory FSL by 3.99 ms, and the duration of the inhibition did not persist longer than the NE tone. n = 10 trials per stimulus.

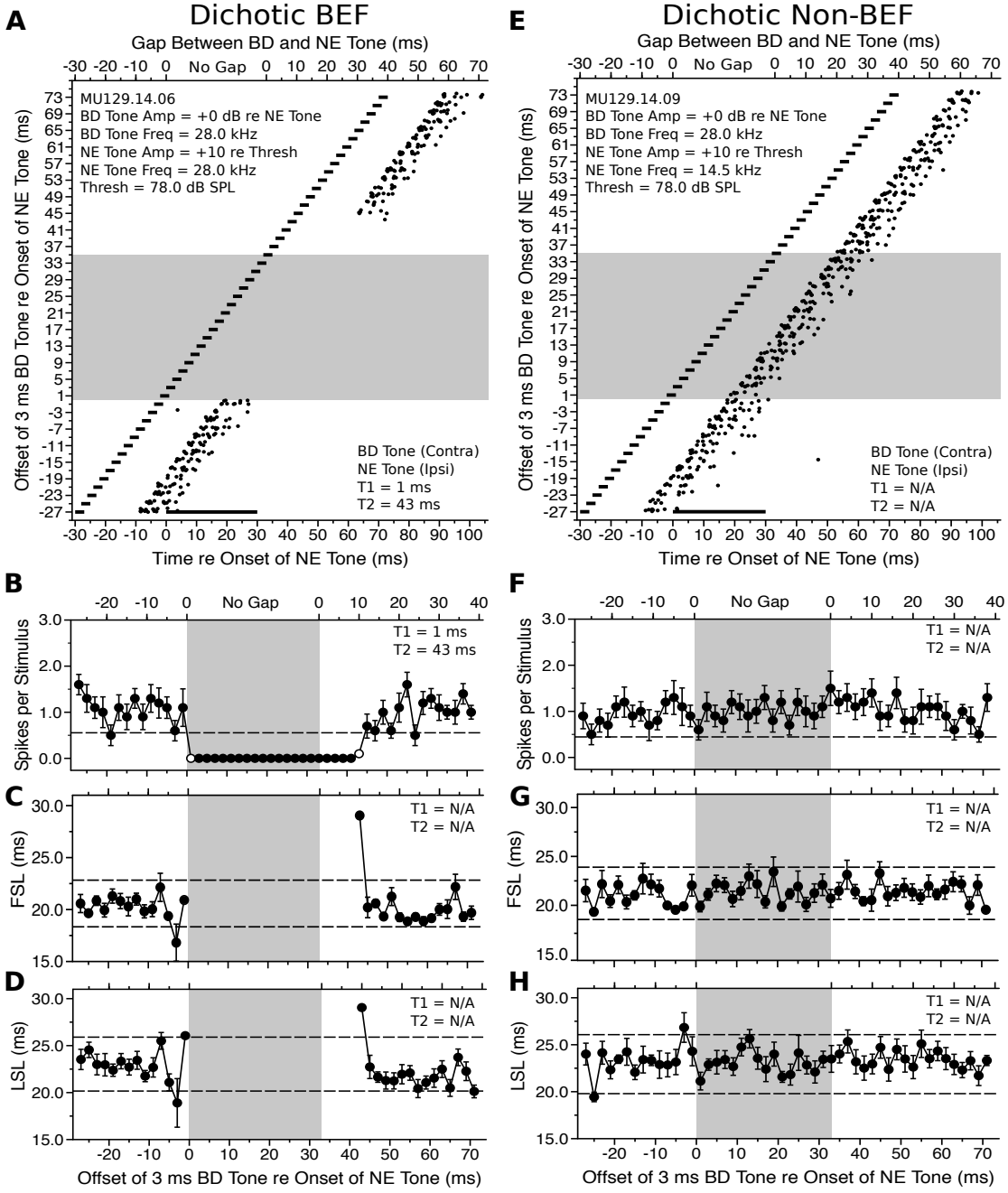
A
Dichotic BEF
Gap Between BD and NE Tone (ms)
MU129.14.06
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 28.0 kHz
NE Tone Amp = +10 re Thresh
NE Tone Freq = 28.0 kHz
Thresh = 78.0 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = 1 ms
T2 = 43 ms
Offset of 3 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
E
Dichotic Non-BEF
Gap Between BD and NE Tone (ms)
MU129.14.09
BD Tone Amp = +0 dB re NE Tone
BD Tone Freq = 28.0 kHz
NE Tone Amp = +10 re Thresh
NE Tone Freq = 14.5 kHz
Thresh = 78.0 dB SPL
BD Tone (Contra)
NE Tone (Ipsi)
T1 = N/A
T2 = N/A
Offset of 3 ms BD Tone re Onset of NE Tone (ms)
Time re Onset of NE Tone (ms)
B
F
Spikes per Stimulus
T1 = 1 ms
T2 = 43 ms
T1 = N/A
T2 = N/A
C
G
FSL (ms)
T1 = N/A
T2 = N/A
D
H
LSL (ms)
T1 = N/A
T2 = N/A
No Gap
Offset of 3 ms BD Tone re Onset of NE Tone (ms)

Fig. 7: Dichotic paired-tone stimulation at the BEF and at a non-BEF outside the 50% eBW. Dot raster displays of responses from a band-pass DTN with the BD tone presented to the contralateral ear and the NE tone presented to the ipsilateral. The contralateral ear was set to the cell's BEF (28.0 kHz; left) and the ipsilateral was set to a non-BEF ($1.5L_{eBW}$) that was outside the eBW (14.5 kHz; right). **A**: When the BD and NE tones were matched at the cells BEF, spike suppression was observed when the 3-ms BD tone and the 30-ms NE tone were sufficiently close in time. **B**: Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones. The first ISI in which spike count dropped to < 50% of baseline was T_1 = 1 ms (leftmost open circle). The last ISI, starting from T_1, when the spike count remained at < 50% of baseline was T_2 = 43 ms (rightmost open circle). **C**: Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The first ISI in which the FSL deviated by $\geq$ 1 SD from baseline was T_1 = - 1 ms (leftmost open circle), and the last ISI when the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 43 ms (rightmost open circle). **D**: Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The first ISI in which the LSL deviated by $\geq$ 1 SD from baseline was T_1 = - 1 ms (leftmost open circle), and the last ISI when the FSL remained deviated by $\geq$ 1 SD from baseline was T_2 = 43 ms (rightmost open circle). In the BEF condition, the final values of T_1 (- 1 ms) and T_2 (22 ms) were determined using LSL and spike count criteria, respectively. In the BEF condition, the onset of the NE tone-evoked inhibition led the excitatory FSL by 1.39 ms, and inhibition persisted 11.39 ms longer than the NE tone. **E**: Dot raster display illustrating responses from the same DTN when the NE tone was presented ipsilaterally at a non-BEF frequency. Spike suppression was not observed with any criteria; there was no change in (**F)** spike count and no deviations in either (**G**) FSL or (**H**) LSL. n = 10 trials per stimulus.

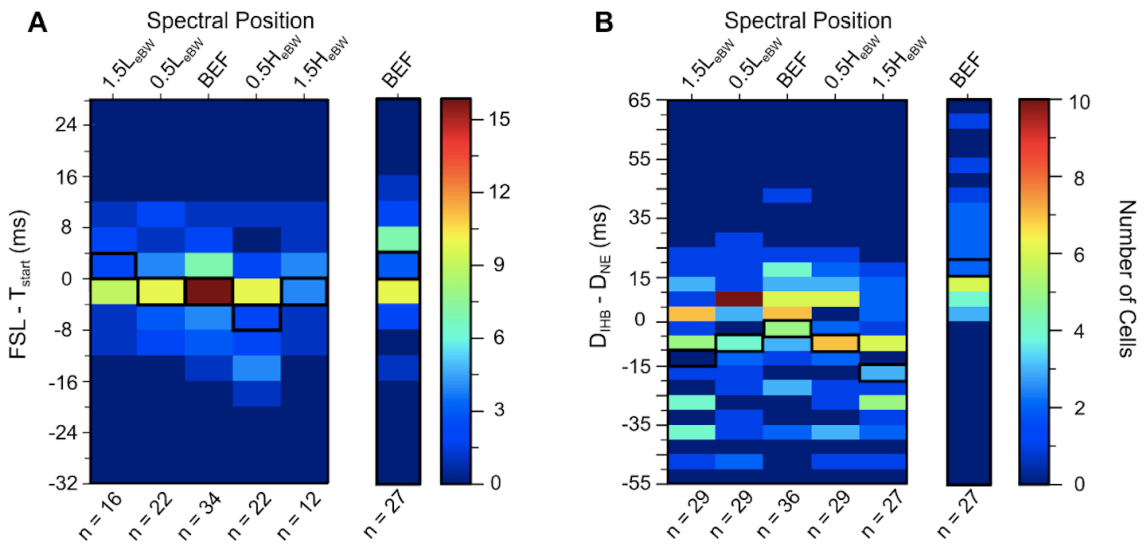

Fig 8: Two-dimensional histograms of the distribution of the duration of leading/lagging and persistent inhibition across the 5 standardized NE tone frequencies for DTNs tested with dichotic paired-tone stimulation (n = 42). Cells tested in the monotic condition (n = 27) when the BD and NE ones were matched to the BEF are also displayed. Mean duration of inhibition at each NE tone frequency is indicated by a black box, with the colour scale showing the proportion of cells 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, and there was no significant difference between the dichotic and monotic conditions at BEF. The cells tested showed leading inhibition in the monotic condition (n = 27), but displayed lagging inhibition (n = 75) 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 $D_{IHB} - D_{NE}$ differed significantly from the monotic condition. Most cells tested in the dichotic condition did not have persistent inhibition, while the same cells tested in the monotic condition almost always had persistent inhibition.

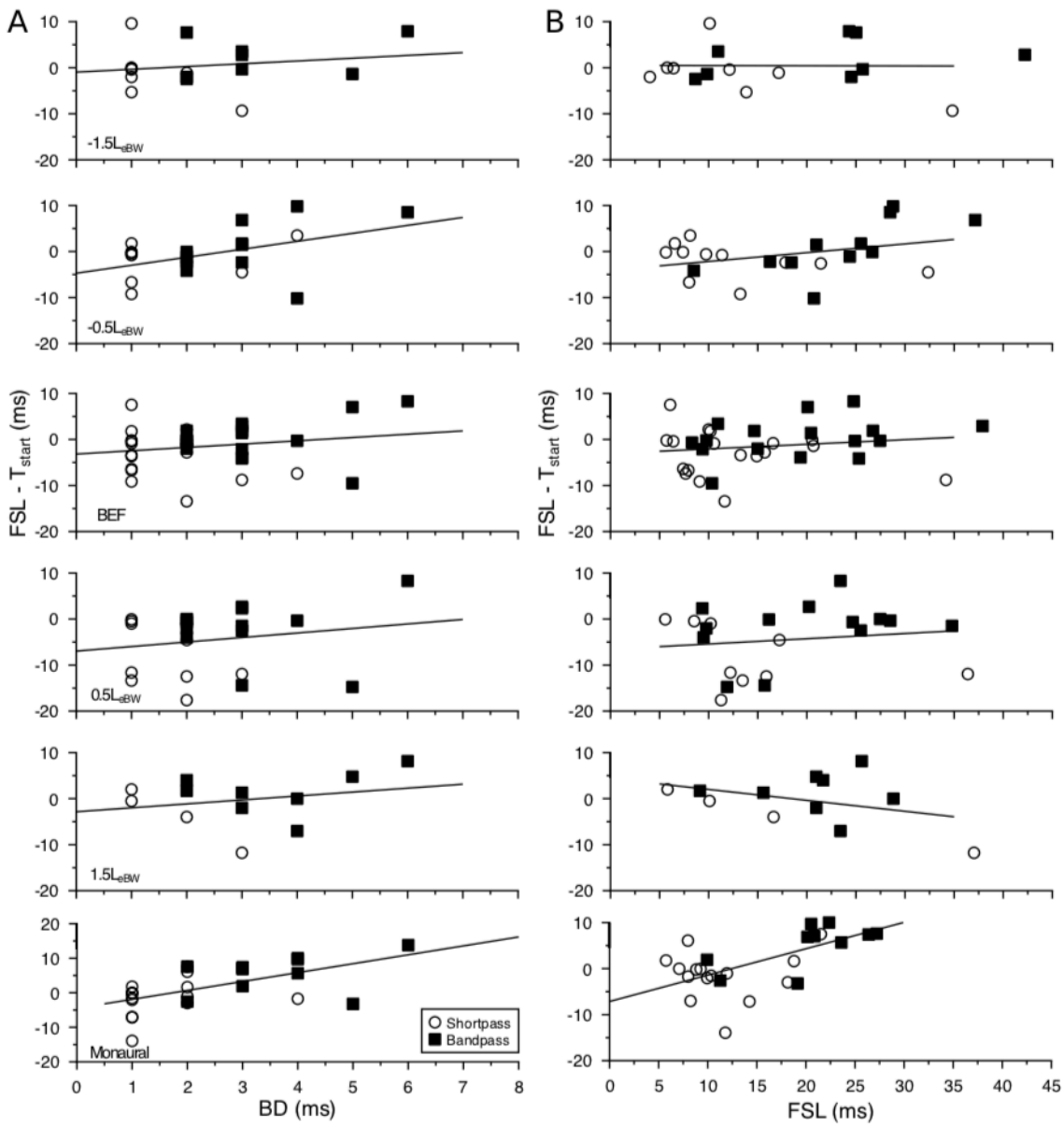

Fig 9: Relation of the duration of leading/lagging inhibition to BD, FSL, and duration filter class. Duration of inhibition was measured with monotic and dichotic paired-tone stimulation at the BEF and at the 5 standardized NE tone frequencies in the dichotic condition and in the monotic condition at BEF. **A**: There was no relation between the leading/lagging inhibition and 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 the two duration filter classes across dichotic conditions. 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 1. Mann-Whitney U-tests for the comparison between duration filter classes are listed in Table 2.

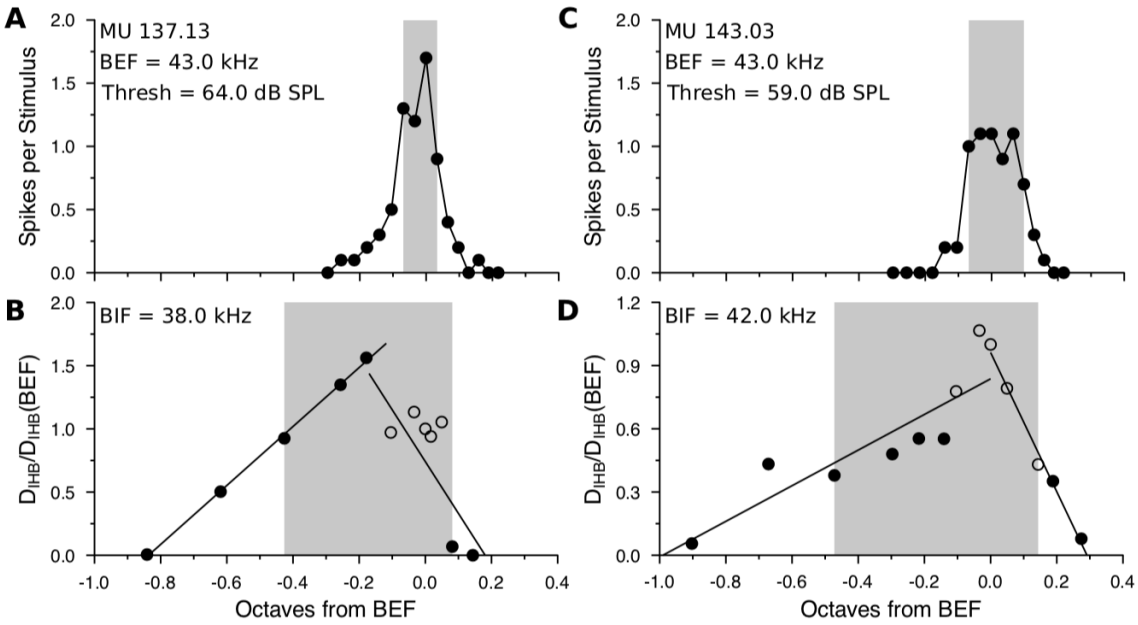

Fig 10: Example BEF, BIF, FRAs, and BWs of 2 short-pass DTNs. The top row show the eFRA of each DTN, plotted as the mean ± SE spike count evoked by varying of BD tone frequencies presented to the contralateral ear. The bottom row displays the iFRAs from the same DTNs, measured during dichotic paired-tone stimulation with variable frequency NE tones presented to the ipsilateral ear and BD tones broadcasted to the contralateral ear. Values are plotted as the normalized duration of inhibition as a function of NE tone frequency in octaves (re BEF). **A**: The eFRA of a short-pass DTN with a BEF of 43.0 kHz and an eBW ranging from 41.0 to 44.0 kHz or -0.068 to 0.033 octaves (re BEF) (grey box). **B**: Normalized iFRA of the same DTN plotted as the duration of inhibition evoked at 12 NE tone frequencies, inside and outside the cell's eBW. For this cell, the BIF was 38.0 kHz and did not match the cell's BEF and the iBW ranged from 32.2 to 45.9 kHz (-0.418 to 0.094 octaves re BEF) (gray box) and was broader that the eBW. **C**: The eFRA of a short-pass DTN with a BEF of 43.0 kHz, and a eBW ranging from 41.0 to 46.0 kHz or -0.069 to 0.097 octaves (re BEF). **D**: Normalized duration of

inhibition evoked at 13 NE tone frequencies inside and outside the cell's eBW. The BIF was 42.0 kHz and closely matched the cell's BEF and the iBW ranged from 30.5 to 47.6 kHz or -0.472 to 0.144 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. The 5 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. All the stimuli presented at +10 dB (re BEF, BD threshold). n = 10 trials per stimulus.

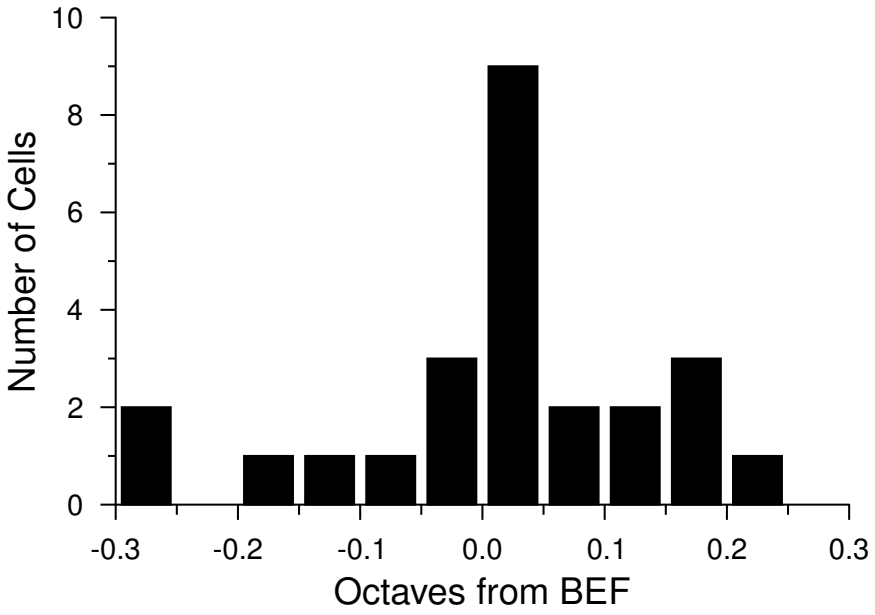

Fig 11: Histogram depicting the difference in octaves between the BEF and BIF in DTNs tested with dichotic paired-tone stimulation. Positive values represent cells with a higher BIF than BEF, and negative values represent cells with a lower BIF than BEF. The majority of DTNs had a BEF that very closely matched its BIF, with 13 cells having a BIF that were within $\pm$ 0.05 octaves or exactly matched (13 cells, 52%). n = 25.

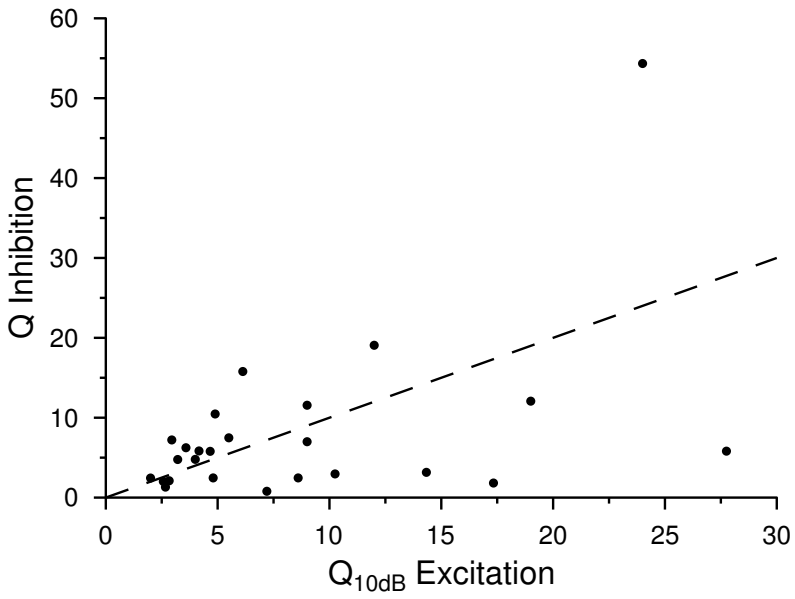

Fig 12: Sharpness of contralateral excitatory and ipsilateral inhibitory tuning in DTNs tested with dichotic paired-tone stimulation. Tuning sharpness plotted as a quality factor (Q). Excitatory tuning sharpness measured as $Q_{10\ dB}$ = BEF/eBW, and inhibitory tuning sharpness measured as Q = BIF/iBW. Data points falling below the y = x identity line represent cells whose iBWs were broader than their eBW. There were 12 cells (48%) that fell below the y=x line and 13 (52%) which were above the y=x line. n = 25 cells.

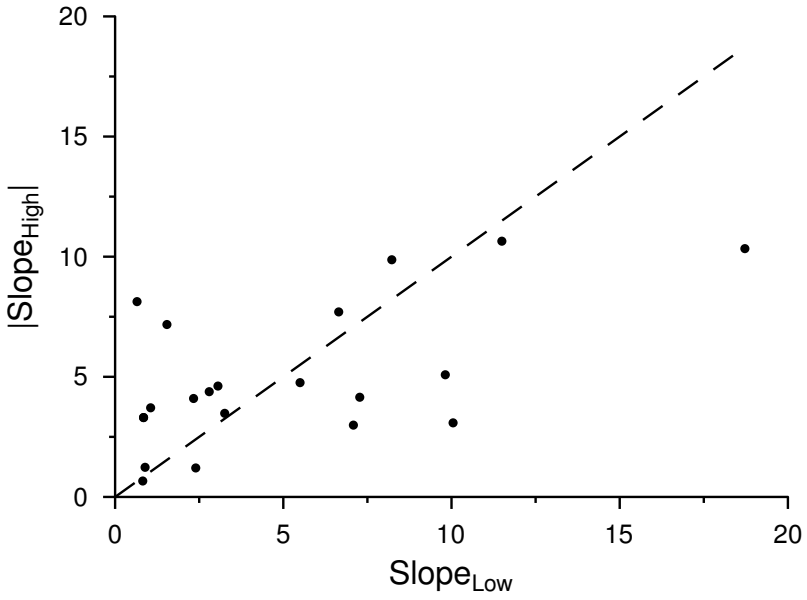

Fig 13: Steepness of the low- and high-frequency tuning slopes measured from the normalized iFRA of DTNs tested with dichotic paired-tone stimulation. Points above the y = x identity line are cells that exhibited an iFRA with a steeper high-frequency slope than low-frequency slope, and points below the 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 low and high-frequency tuning slopes. There were 9 cells (43%) that fell below the y=x line and 12 cells (57%) that were above the y=x line. n = 21 cells.

Table 1. Relation of duration of inhibition ($L_{First} - T_{Start}$) to BD and FSL for DTNs tested with monotic and dichotic paired-tone stimulation. Statistically significant correlations are indicated in boldface. The only significant correlations were in the monotic conditions when the BD and NE tones were presented monaurally and both tones were matched to the cell's BEF.

Comparison	Frequency	n	Slope	Intercept	R^2	P-Value
$L_{first} - T_{start}$ (ms) vs. BD (ms)	Monotic	26	2.5809	- 4.4892	0.3388	**0.002***
	$1.5L_{eBW}$	16	0.2716	- 0.4748	0.0138	0.497
	$0.5L_{eBW}$	22	0.8913	- 2.5049	0.1089	0.031
	BEF	34	0.519	- 2.3991	0.0246	0.278
	$0.5H_{eBW}$	22	0.7138	- 4.9564	0.0279	0.416
	$1.5H_{eBW}$	12	0.317	- 0.9284	0.0205	0.446

Comparison	Frequency	n	Slope	Intercept	R^2	P-Value
$L_{first} - T_{start}$ (ms) vs. FSL (ms)	Monotic	27	0.5731	- 7.1448	0.3914	**0.001***
	$1.5L_{eBW}$	16	0.2716	- 0.4748	0.0138	0.971
	$0.5L_{eBW}$	22	0.1203	- 2.3482	0.0738	0.104
	BEF	34	0.0719	- 2.297	0.0184	0.336
	$0.5H_{eBW}$	22	0.0399	- 3.8004	0.0037	0.525
	$1.5H_{eBW}$	12	- 0.0802	1.2456	0.0535	0.211

* Significant differences were determined by a Bonferroni corrected family-wise error rate of $\alpha < 0.008$

Table 2. Duration of inhibition ($L_{First} - T_{Start}$) in short-pass and band-pass DTNs tested with monotic paired-tone stimulation at the BEF and dichotic paired-tone stimulation at the 5 standardized NE tone frequencies (re 50% eBW). Statistically significant differences are indicated in boldface. Sample sizes at each frequency are: n = 16 ($1.5L_{eBW}$), n = 22 ($0.5L_{eBW}$), n = 34 (BEF), n = 22 ($0.5H_{eBW}$), n = 12 ($1.5H_{eBW}$), n = 27 (monotic)

Condition	Duration Filter Class	Mean ± SD (ms)	U	P-Value
Monotic	Short-pass	1.39 ± 5.24	30.00	**0.005***
	Band-pass	5.83 ± 5.21		
Dichotic				
$1.5L_{EBW}$	Short-pass	-1.08 ± 4.18	23.50	0.382
	Band-pass	1.97 ± 5.40		
$0.5L_{EBW}$	Short-pass	-1.99 ± 3.68	44.00	0.300
	Band-pass	0.74 ± 5.89		
BEF	Short-pass	-3.00 ± 4.97	89.00	0.059
	Band-pass	0.22 ± 4.31		
$0.5H_{EBW}$	Short-pass	-8.12 ± 6.62	32.00	0.082
	Band-pass	-2.08 ± 6.32		
$1.5H_{EBW}$	Short-pass	-3.56 ± 5.99	8.00	0.214
	Band-pass	1.35 ± 4.61		