Monaural and Binaural Inhibition Underlying Duration-Tuned Neurons in the Inferior Colliculus

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Duration-tuned neurons (DTNs) in the mammalian inferior colliculus (IC) arise from a combination of excitatory and inhibitory synaptic inputs. Previous research has shown that the inhibition responsible for creating DTNs has a shorter latency than that of excitation and lasts longer than the stimulus duration. We used monotic and dichotic paired tone stimulation and recorded responses of DTNs from the IC of the bat to assess the relative contributions of each ear in forming duration-tuned circuits. The stimulus consisted of a short best duration (BD) excitatory tone and a longer duration nonexcitatory (NE) tone. In the monotic condition, when the BD and NE tones were presented to the contralateral ear and were sufficiently close in time, the NE tone always suppressed spikes evoked by the BD tone. In the dichotic condition, when the BD tone was presented to the contralateral ear and the NE tone to the ipsilateral ear, half of DTNs no longer showed spike suppression to the NE tone. Of those DTNs with suppression in both conditions, the latency of the inhibition was shorter and the duration of the inhibition was longer in the monotic condition. Therefore, in the monotic condition, DTNs received a contralaterally evoked inhibitory input that preceded the excitatory input to the same neuron. In the dichotic condition, DTNs received an ipsilaterally evoked inhibitory input that was weaker, longer in latency, and shorter in duration than the inputs from the contralateral ear. These findings indicate that the neural mechanisms that create DTNs in the IC are monaural.

Key words: audition; auditory midbrain; big brown bat (*Eptesicus fuscus*); hearing; paired tone stimulation; temporal masking

Introduction

The auditory midbrain or inferior colliculus (IC) is the first nucleus in the central auditory pathway that contains neurons with duration-selective responses. These duration-tuned neurons (DTNs) are found in the IC and auditory cortex in a variety of vertebrates. Although DTNs have been primarily studied in bats and are likely important for echolocation, they have also been reported from the auditory midbrain of non-echolocating amphibians and mammals (for review, see Sayegh et al., 2011). Therefore, the role of DTNs in normal hearing cannot be exclusive for echolocation. Although their exact functions are unknown, DTNs provide a potential neural mechanism for processing temporal features of sound in human speech (Denes, 1955) and species-specific communication sounds (Pollack and Hoy, 1979; Bohn et al., 2008; Gadziola et al., 2012).

Intracellular (Covey et al., 1996; Tan and Borst, 2007; Leary et al., 2008) and extracellular (Casseday et al., 1994, 2000; Ehrlich et al., 1997; Chen, 1998; Faure et al., 2003) recordings from DTNs have led to conceptual models of the neural mechanism(s) responsible for duration selectivity based on the interaction of excitatory and inhibitory synaptic inputs offset in time (Casseday et al., 1994, 2000; Fuzessery and Hall, 1999; Sayegh et al., 2011). Indeed, computational models of these mechanisms can reproduce the *in vivo* spiking responses of DTNs in both echolocating bats and non-echolocating vertebrates (Aubie et al., 2009, 2012).

Paired tone stimulation combined with single-unit extracellular recording has been used previously to characterize the strength and time course of inhibitory inputs to DTNs (Faure et al., 2003). These experiments were conducted with monotic stimulation to the contralateral ear. To characterize the relative contributions of each ear in forming and modifying duration-selective responses, we used monotic and dichotic paired tone stimulation, with one excitatory tone set to the best duration (BD) of the neuron and another tone set to a longer, nonexcitatory (NE) duration. In both conditions, the BD tone was presented to the contralateral ear. In the monotic condition, the NE tone was also presented to the contralateral (excitatory) ear, whereas in the dichotic condition, the NE tone was presented to the ipsilateral (inhibitory) ear. Using this stimulus paradigm, we found that monotic paired tone stimulation always caused spike suppression in DTNs via sustained inhibition evoked by the NE tone; however, during dichotic paired tone stimulation, approximately half of the cells were not suppressed by the NE tone. We

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expected to see a greater inhibitory effect in the dichotic condition because ipsilateral ear stimulation typically suppresses contralaterally evoked spikes of IC neurons (Hind et al., 1963; Rose et al., 1966). For those DTNs with binaural inhibition, the inhibition evoked in the dichotic condition was weaker in strength, shorter in duration, and delayed relative to the inhibition evoked monaurally. Altogether, these results support the conclusion that the underlying neural mechanisms that create DTNs in the mammalian IC are primarily monaural.

Materials and Methods

Surgical procedures. Methods for surgical procedures and electrophysiological recordings have been described previously (Faure et al., 2003; Aubie et al., 2012; Sayegh et al., 2012). Briefly, electrophysiological recordings were obtained from 15 big brown bats (*Eptesicus fuscus*) of both sexes at the University of Washington and McMaster University. To facilitate multiple recordings from individual animals and to precisely replicate the position of the bat's head between recording sessions, a stainless steel post was glued to the skull. Before surgery, bats were given a subcutaneous injection of buprenorphine (0.03 ml; 0.025 mg/kg). For surgery, bats were first placed in an anesthesia induction chamber (12 × 10 × 10 cm) where they inhaled a 1–5% isoflurane/oxygen mixture (1 L/min). Anesthetized bats were then placed in a foam-lined body restraint within a model 1900 stereotaxic alignment system with a custom bite bar and gas mask (David Kopf Instruments) for continuous isoflurane inhalation. The hair covering the skull was cropped and the skin disinfected with Betadine surgical scrub. Local anesthetic (0.2 ml of bupivacaine; 5 mg/ml) was injected subcutaneously before making a midline incision in the scalp. The temporal muscles were reflected, the skull was scraped clean and swabbed with 70–100% ethanol, and a post was glued to the skull overlying the cortex with cyanoacrylate adhesive (Loc-tite; Henkel) cured with liquid acrylic hardener (Zipkicker; Pacer Technology). A chlorided silver wire attached to the head post was placed under the temporal muscles and served as the reference electrode. Recordings began 1–4 d after surgery. Each bat was used in one to eight sessions lasting 6–8 h each. Recordings were terminated if a bat showed signs of discomfort. Between sessions, the electrode penetration site was covered with a piece of contact lens and Gelfoam coated in Polysporin. Bats were housed individually in a temperature- and humidity-controlled room. All procedures were approved by the University of Washington Laboratory Animal Care and Use Committee or the McMaster University Animal Research Ethics Board and were in accordance with guidelines published by the National Institutes of Health and the Canadian Council on Animal Care.

Electrophysiological recordings. Electrophysiological recordings were conducted inside a double-walled, sound-attenuating booth with electrical shielding (Industrial Acoustics). Before recording, each bat was given a subcutaneous injection of neuroleptic (0.3 ml; 1:1 mixture of 0.05 mg/ml fentanyl citrate and 2.5 mg/ml droperidol; 19.1 mg/kg). Bats were then placed in a foam-lined body restraint that was suspended by springs within a small animal stereotaxic frame customized for bats (ASI Instruments) and mounted atop an air vibration table (TMC Micro-g). The bat's head was immobilized by securing the head post to a stainless steel rod attached to a micromanipulator (ASI Instruments) mounted on the stereotaxic frame. The dorsal surface of the IC was exposed for recording by making a small opening in the skull and dura mater with a scalpel. Single-unit extracellular recordings were made with thin-wall borosilicate glass microelectrodes with a capillary filament (1.2 mm outer diameter; A-M Systems) and filled with 0.3–3 M NaCl or physiological saline. Typical electrode resistances ranged from 10 to 30 MΩ. Electrodes were manually positioned over the dorsal surface of the IC with a manipulator (ASI Instruments) and advanced into the brain with a stepping hydraulic micropositioner (model 2650; David Kopf Instruments). Action potentials were recorded with a Neuroprobe amplifier (model 1600; A-M Systems), the 10× output of which was bandpass filtered and further amplified (500–1000×) by a Tucker Davis Technologies (TDT) spike preconditioner (TDT PC1; low-pass $f_c = 7$ kHz; high-pass $f_c = 300$ Hz). Spike times were logged on 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).

Stimulus generation. Stimulus generation and online data collection were controlled with custom software that displayed spike times as peristimulus dot raster displays (rastergrams) ordered by the acoustic parameter that was varied (Faure et al., 2003). Sound pulses were digitally generated with a two-channel array processor (TDT Apos II; 357 kHz sampling rate) optically interfaced to two digital-to-analog converters (TDT DA3-2) whose individual outputs were fed to a two-channel low-pass anti-aliasing filter (TDT FT6-2; $f_c = 120$ kHz), two programmable attenuators (TDT PA5), and two signal mixers (TDT SM5) with equal weighting. The output of each mixer was fed to a manual attenuator (Leader LAT-45) before final amplification (model 7500; Krohn-Hite). Auditory stimuli were presented via a pair of Brüel & Kjær one-quarter-inch condenser microphones (type 4939; protective grid on), modified for use as loudspeakers with a transmitting adaptor (type UA-9020; Brüel & Kjær) to correct for nonlinearities in the transfer function (Frederiksen, 1977). Each loudspeaker was positioned ~1 mm in front of the external auditory meatus. The output of each speaker, measured with a Brüel & Kjær type 4138 one-eighth-inch condenser microphone (90° incidence; grid off) connected to a measuring amplifier (type 2606; Brüel & Kjær) and bandpass filter (model 3500; Krohn-Hite), was quantified with a sound calibrator (type 4231; Brüel & Kjær) and expressed in decibels sound pressure level (dB SPL relative to 20 μPa) equivalent to the peak amplitude of continuous tones of the same carrier frequency. The loudspeaker transfer function was flat ±6 dB from 28 to 118 kHz, and there was at least 30 dB attenuation at the ear opposite the source (Ehrlich et al., 1997). All stimuli had rise/fall times of 0.4 ms shaped with a square cosine function and were presented at a rate of 3 Hz. Search stimuli were presented monaurally, contralateral to the IC being recorded. Single units were found by presenting short-duration pure tones and/or downward frequency modulated sweeps. During unit isolation, we determined the characteristic frequency, minimum threshold, and first-spike latency (relative to signal onset) for each cell, and for DTNs, we also determined the BD and filter response class (for definitions, see Faure et al., 2003; Fremouw et al., 2005; i.e., short-pass, bandpass, or long-pass DTN).

Following the paired tone stimulation paradigms of Faure et al. (2003) and Covey and Faure (2005), we presented DTNs with pairs of characteristic frequency tones that differed in duration and interstimulus interval (ISI). One tone in the pair was used as a probe and was set to the BD (BD tone; D_{BD}); the other was as NE tone set to a longer duration that did not evoke spikes (NE tone; D_{NE}). The duration of the NE tone was typically set to 10× the duration of the BD tone. The onset time of the NE tone was fixed between trials, whereas the onset time of the BD tone was randomly varied in 2 ms steps. Unless stated otherwise, the BD and NE tones were matched in frequency, amplitude, and phase at all ISIs. The frequency of the BD and NE tones was set to the characteristic frequency of the cell. The amplitude of the BD tone was typically set to +10 dB (relative to threshold), and the amplitude of the NE tone was set to +0, +10, or +20 dB (relative to BD tone). Because the BD and NE tones were matched in frequency and phase, whenever the tones overlapped they summed to form a single composite tone with a +6 dB amplitude pedestal, the duration of which was determined by the amount of temporal overlap.

Determining the time course of inhibition. For each cell, we measured the latency and duration of the inhibition evoked by the stationary NE tone during monotic and dichotic paired tone stimulation by observing the times when spikes evoked by the roving BD tone were suppressed and/or altered in latency. In both conditions, the BD tone was presented to the contralateral (excitatory) ear. In the monotic condition, the NE tone was electronically mixed and also presented to the contralateral ear, whereas in the dichotic condition, the NE tone was split and presented to the ipsilateral (inhibitory) ear while the BD tone was presented to the contralateral (excitatory) ear. In this way, we were able to measure and compare the net inhibition evoked by the NE tone through the monaural and binaural central auditory pathways.

Figure 1 is an example of spiking responses evoked from a bandpass DTN tested with monotic (monaural) paired tone stimulation using

Figure 1. Measuring the time course of inhibition with paired tone stimulation. **A**, Dot raster display illustrating how changes in the number and timing of action potentials were used to calculate the T_1 and T_2 of the inhibition evoked by the NE tone using the equations given at the bottom. The BD and NE tones are illustrated with black bars, short bars represent the roving 3 ms BD tone, and a single long bar represents the stationary 30 ms NE tone. On each trial, the cell was presented with a BD and NE tone that were randomly varied in ISI (for clarity, the NE tone is drawn only once). There were 15 stimulus repetitions per ISI tested. The gray box indicates the range of times over which the BD and NE tones were temporally contiguous or overlapping. Note the gap in the response of the cell when the BD and NE tones were sufficiently close in time. Circled responses are the 10 trials that were used to calculate the mean $\pm$ SD baseline spike count (1.70 ± 1.02 spikes per stimulus), baseline FSL ($L_{\text{first}} = 13.85 \pm 1.19$ ms relative to BD tone onset), and the baseline LSL ($L_{\text{last}} = 16.09 \pm 1.40$ ms relative to BD tone onset). For this cell, the first ISI with a significant deviation from the baseline spike count or latency was $T_1 = -5$ ms (determined by LSL), and the longest ISI with a significant deviation from the baseline spike count or latency was $T_2 = 37$ ms (consensus from all measures). The calculated inhibition $T_{\text{start}} = 8.09$ ms and the inhibition $T_{\text{end}} = 47.85$ ms, resulting in an effective duration of inhibition of 39.76 ms. The cell had 5.76 ms of leading inhibition and 9.76 ms of persistent inhibition (see Materials and Methods). **B**, Mean $\pm$ SE spikes per stimulus as a function of the offset of the BD tone relative to the onset of the NE tone. The dotted line represents 50% of the baseline spike count. The leftmost open circle is the first ISI in which the spike count dropped to $\leq 50\%$ of baseline with a consecutive data point also $\leq 50\%$ of baseline; this point represents the onset of spike suppression measured with spike counts ($T_1 = -3$ ms). The rightmost open circle represents the longest ISI, starting from T_1 , in which the spike count was still $\leq 50\%$ of baseline and with the two next consecutive data points $> 50\%$ of baseline; this point represents the offset of spike suppression measured with spike counts ($T_2 = 37$ ms). **C**, Mean $\pm$ SE FSL as a function of the offset of the BD tone relative to the onset of the NE tone. Dotted lines represent ± 1 SD relative to baseline FSL. The leftmost open circle is the first ISI with a FSL that deviated by > 1 SD from baseline with a consecutive data point also > 1 SD from baseline; this point represents the onset of spike suppression measured with FSL ($T_1 = 25$ ms). The rightmost open circle is the longest ISI, starting from T_1 , in which the FSL deviated by > 1 SD from baseline with the two next consecutive data points within 1 SD of baseline; this point represents the offset of spike suppression measured with FSL ($T_2 = 37$ ms). **D**, Mean $\pm$ SE LSL as a function of the offset of the BD tone relative to the onset of the NE tone. Dotted lines represent ± 1 SD relative to baseline LSL. The leftmost open circle is the first ISI with a LSL that deviated by > 1 SD from baseline with a consecutive data point also > 1 SD from baseline; this point represents the onset of spike suppression measured with LSL ($T_1 = -5$ ms). The rightmost open circle is the longest ISI, starting from T_1 , in which the LSL deviated by > 1 SD from baseline with the two next consecutive data points within 1 SD of baseline; this point represents the offset of spike suppression measured with LSL ($T_2 = 37$ ms). Amp, Amplitude; Contra, contralateral; Freq, frequency; Thresh, threshold.

equal-amplitude BD and NE tones. Also illustrated are details of how we measured the onset (T_1) and offset (T_2) of spike suppression using changes in the spike count, first spike latency (FSL), and/or last spike latency (LSL) of the cell. Note the suppression in spiking when the BD and NE tones were presented at sufficiently small ISIs. The circled responses represent the 10 trials with the largest ISIs, when the BD tone preceded the NE tone, that were used to calculate the mean $\pm$ SD baseline spike count and baseline FSL and LSL (Fig. 1A). For this cell, the baseline spike count was 1.70 ± 1.02 spikes per stimulus, the baseline FSL (L_{first}) was 13.85 ± 1.19 ms (relative to BD tone onset), and the baseline LSL (L_{last}) was 16.09 ± 1.40 ms (relative to BD tone onset).

For each cell, we plotted the average spike count, FSL, and LSL as a function of the gap between the offset of the BD tone and the onset of the NE tone (Fig. 1B–D). We then used changes in the evoked response to

measure the onset (latency) and time course (duration) of spike suppression, and thus the neural inhibition, evoked by the NE tone. Starting from the largest ISI when the BD tone preceded the NE tone, the onset (T_1) of spike suppression was defined to be the first data point at which the spike count decreased to $\leq 50\%$ of the baseline count (or the spike latency increased or decreased by > 1 SD from the baseline latency) as long as the following data point also remained $\leq 50\%$ (or > 1 SD) from baseline (Sayegh et al., 2012). The offset (T_2) of spike suppression was defined to be the longest ISI, starting from T_1 and moving toward ISIs when the BD tone followed the NE tone, in which the spike count (or latency) remained $\leq 50\%$ (or deviated by > 1 SD) from baseline if the two consecutive data points were $> 50\%$ (or within ± 1 SD) of baseline. Using spike count criteria on the bandpass DTN in Figure 1, the onset and offset times of spike suppression were $T_1 = -3$ ms and $T_2 = 37$ ms, respectively

(Fig. 1B). Using spike latency criteria, the onset and offset times of spike suppression measured with FSL changes were $T_1 = 25$ ms and $T_2 = 37$ ms, respectively (Fig. 1C), whereas using LSL changes, they were $T_1 = -5$ ms and $T_2 = 37$ ms, respectively (Fig. 1D).

The final values for T_1 and T_2 were chosen using the most sensitive criteria that reflected the time course of spike suppression (see below, Comparing inhibition measured with spike counts and latencies). For the bandpass DTN in Figure 1, the final value for T_1 was -5 ms and was chosen from the LSL measure, and the final value for T_2 was -37 ms because there was consensus across the spike count and latency measures. After selecting these values, the effective start (T_{start}) and end (T_{end}) times of the neural inhibition evoked by the NE tone were calculated as follows:

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

and

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

where L_{last} was the baseline LSL, L_{first} was the baseline FSL, and D_{BD} was the duration of the BD tone. The effective duration of inhibition was calculated as $T_{\text{end}} - T_{\text{start}}$.

A neuron was said to have leading inhibition when the latency of the inhibition evoked by the NE tone was less than the excitatory FSL of the cell (i.e., $T_{\text{start}} < L_{\text{first}}$). A neuron was said to have persistent inhibition when the effective duration of the inhibition evoked by the NE tone was greater than the duration of the NE tone (i.e., $T_{\text{end}} - T_{\text{start}} > D_{\text{NE}}$). For the bandpass neuron in Figure 1, $T_{\text{start}} = 8.09$ ms and $T_{\text{end}} = 47.85$ ms, resulting in an effective duration of inhibition of 39.76 ms. This cell had 5.76 ms of leading inhibition because the latency of the inhibition evoked by the NE tone ($T_{\text{start}} = 8.09$ ms) was shorter than the excitatory baseline FSL of the cell ($L_{\text{first}} = 13.85$ ms). The cell also had persistent inhibition because the effective duration of the inhibition ($T_{\text{end}} - T_{\text{start}} = 39.76$ ms) was 9.76 ms longer than the duration of the NE tone ($D_{\text{NE}} = 30$ ms).

Comparing inhibition measured with spike counts and latencies. The final values for T_1 and T_2 could be chosen from a combination of spike count and latency measures because doing so increased the sensitivity for quantifying the suppressive effects of the NE tone on the spiking responses evoked by the BD tone (Faure et al., 2003; Sayegh et al., 2012). In cases in which cells responded with only a single spike per stimulus (i.e., $L_{\text{first}} = L_{\text{last}}$) or in instances in which the spike count of the cell was clearly suppressed although L_{first} or L_{last} (or both) remained within ± 1 SD of baseline, a change in spike count was typically used for selecting T_1 and T_2 because this criterion was usually more sensitive in reflecting the time course of the suppression. In cases in which cells responded with more than one spike per stimulus or in instances in which the spike count of the cell had recovered to within 50% of baseline although 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 usually more sensitive in reflecting the time course of the suppression. In cases in which the mean spike count or latency had not returned to within 50% or ± 1 SD of baseline, respectively, over the range of ISIs presented (monotic condition, $n = 10$ cells; dichotic condition, $n = 0$ cells), T_2 was conservatively estimated as the longest ISI tested.

In the monotic paired tone stimulation condition, there was consensus between spike count and spike latency criteria for the final value of T_1 in 17 of 42 cells (40.4%). In the remaining 25 cells, spike counts were used in 17 cases and spike latencies were used in eight cases (seven with LSL and one with FSL). The mean $\pm$ SD spike count for the 17 cells analyzed with a spike count criterion was 1.17 ± 0.42 spikes per stimulus, and for the eight cells analyzed with a spike latency criterion, it was 1.88 ± 0.63 spikes per stimulus; spike counts in these two groups were significantly different (Mann–Whitney $U = 120.00$, $p = 0.01$). For the final value of T_2 , there was agreement between spike count and spike latency criteria for 5 of 42 cells (11.9%). In the remaining 37 cells, spike counts were used in 20 cases, and spike latencies were used in 17 cases (14 with FSL and three with LSL). The mean $\pm$ SD spike count for the 20 cells analyzed with a spike count criterion was 1.14 ± 0.39 spikes per stimulus, and for the 17 cells analyzed with a spike latency criterion, it was 1.84 ± 0.58

spikes per stimulus; spike counts in these two groups were also significantly different ($t_{(35)} = -4.319$, $p \ll 0.001$).

In the dichotic condition, there was consensus between spike count and spike latency criteria for the final T_1 value in 3 of 20 cells (15.0%). In the remaining 17 cells, spike counts were used in nine cases, and spike latencies were used in eight cases (three with LSL, four with FSL, and one consensus). The mean $\pm$ SD spike count for the nine cells analyzed with a spike count criterion was 1.29 ± 0.51 spikes per stimulus, and for the eight cells analyzed with a spike latency criterion, it was 2.27 ± 0.69 spikes per stimulus; spike counts in these two groups were significantly different (Mann–Whitney $U = 62.00$, $p = 0.011$). For the final value of T_2 , spike count and spike latency criteria were in agreement for 5 of 20 cells (25.0%). In the remaining 15 cells, spike counts were used in five cases and spike latencies were used in 10 cases (eight with FSL, one with LSL, and one consensus). The mean $\pm$ SD spike count for the five cells analyzed with a spike count criterion was 1.44 ± 0.65 spikes per stimulus, and for the 10 cells analyzed with a spike latency criterion, it was 1.63 ± 0.76 spikes per stimulus; spike counts in these two groups were not significantly different (Mann–Whitney $U = 27.00$, $p = 1.00$).

We compared the effective duration of inhibition ($T_{\text{end}} - T_{\text{start}}$) measured with spike counts and spike latencies in 37 DTNs tested with monotic paired tone stimulation. The mean $\pm$ SD effective duration of inhibition was 37.13 ± 15.15 ms when measured with spike counts and 33.89 ± 15.58 ms when measured with spike latencies. The difference between the two measures was not statistically significant (Wilcoxon's signed-rank test, $Z = -1.241$, $p = 0.214$). This analysis validates that changes in spike counts or latencies can be used to quantify the time course of spike suppression evoked by the NE tone.

Data analyses. Unless stated otherwise, all data are reported as the mean $\pm$ SE. Data were tested for normality and homogeneity of variances with Shapiro–Wilk's and Levene's tests, respectively, before additional statistical analyses. Parametric tests were used when data were normally distributed with equal variances; otherwise, an equivalent nonparametric test was used. The proportion of DTNs with measurable inhibition in the monotic and dichotic paired tone conditions was compared with a Fisher's exact test. Two-sample t tests or Mann–Whitney U tests were used to compare evoked spike counts between cells in which the timing of suppression was measured with spike count or spike latency criteria. The latency and time course of the inhibition evoked in the monotic and dichotic conditions, and the durations of inhibition measured with spike counts and latencies, were compared with paired t tests or Wilcoxon's signed-rank tests. Linear regressions were calculated to evaluate the relationship of BD, FSL, and leading/lagging inhibition. The effect of increasing the amplitude of the NE tone in the dichotic condition was evaluated with a Friedman's test. All statistical analyses were performed in SPSS or Python (SciPy) and used an experiment-wise error rate of $\alpha = 0.05$.

Results

Inhibition evoked with monotic and dichotic paired tone stimulation

Figure 2 shows an example of responses evoked from a short-pass DTN tested with monotic and dichotic paired tone stimulation using equal-amplitude BD and NE tones. In this cell, spike suppression was observed for the monotic condition but was absent for the dichotic condition. In the monotic condition, when the 1 ms BD tone and the 10 ms NE tone were broadcast to the contralateral ear (Fig. 2A), there was a pronounced reduction in the spike count (Fig. 2B) and a significant deviation in both the FSL and LSL (Fig. 2C,D). The final value for T_1 was 1 ms and was derived from a change in spike count; the final value for T_2 was 49 ms and was derived from a change in FSL. The effective duration of spike suppression was calculated to be 47.21 ms. Note that this neuron did not show leading inhibition because $L_{\text{first}} = 8.81$ ms and $T_{\text{start}} = 9.60$ ms, thus inhibition lagged excitation by 0.79 ms; however, it may be that the excitatory and inhibitory inputs to this cell were coincident because the difference in latency between

Figure 2. Example 1, Comparing monotic and dichotic paired tone stimulation. Dot raster display illustrating spike suppression in a short-pass DTN in response to monotic (left) but not dichotic (right) paired tone stimulation. **A**, When both tones were presented to the contralateral ear, spike suppression occurred when the 1 ms BD tone and the 10 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 shortest ISI in which the spike count was $\leq 50\%$ of baseline was $T_1 = 1$ ms. The longest ISI, starting from T_1 , in which the spike count was $\leq 50\%$ of baseline was $T_2 = 25$ ms. **C**, Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The shortest ISI in which the FSL deviated by >1 SD from baseline was $T_1 = 3$ ms, and the longest ISI in which the FSL deviated by >1 SD from baseline was $T_2 = 49$ ms. **D**, Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The shortest and longest ISIs in which the LSL deviated by >1 SD from baseline was $T_1 = T_2 = 3$ ms. In the monotic condition, the earliest T_1 time was 1 ms, determined with spike counts, and the longest T_2 time was 49 ms, determined with FSL. In the monotic condition, the latency of inhibition lagged behind the excitatory FSL by 0.79 ms, and the inhibition persisted 37.21 ms longer than the NE tone. **E**, Dot raster display illustrating the responses of the same DTN to dichotic paired tone stimulation (BD tone to contralateral ear; NE tone to ipsilateral ear). There was no significant change in the spike count (**F**), FSL (**G**), or LSL (**H**) of the cell. This example shows no evidence of ipsilaterally evoked inhibition; therefore, the inhibition that created the duration-selective response of the neuron was purely monaural and evoked by stimulation of the contralateral ear. Contra, Contralateral; Freq, frequency; Ipsi, ipsilateral; Thresh, threshold.

excitation and inhibition was within the measurement resolution (2 ms) of the stimulus paradigm. The cell had strong persistent inhibition, revealed by changes in both its spike count (Fig. 2B) and FSL (Fig. 2C), because the duration of spike suppression lasted 37.21 ms longer than the 10 ms NE tone.

In the dichotic condition, spike suppression was no longer observed when the BD tone was presented to the contralateral ear and the NE tone was presented to the ipsilateral ear (Fig. 2E). At all ISIs, the spike count remained $\geq 50\%$ of baseline (Fig. 2F), and neither the FSL nor the LSL deviated by >1 SD from baseline over two consecutive ISIs (Fig. 2G,H).

Figure 3. Example 2, Comparing monotic and dichotic paired tone stimulation. Dot raster display illustrating spike suppression in a bandpass DTN in response to monotic (left) and dichotic (right) paired tone stimulation. **A**, When both tones were presented to the contralateral ear, spike suppression occurred 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 was $\leq 50\%$ of baseline was $T_1 = -4$ ms. The longest ISI, starting from T_1 , in which the spike count was $\leq 50\%$ of baseline was $T_2 = 26$ ms. **C**, Mean $\pm$ SE FSL as a function of the ISI between the BD and NE tones. The shortest ISI in which the FSL deviated by >1 SD from baseline was $T_1 = 18$ ms, and the longest ISI in which the FSL deviated by >1 SD from baseline was $T_2 = 20$ ms. **D**, Mean $\pm$ SE LSL as a function of the ISI between the BD and NE tones. The shortest and longest ISIs in which the LSL deviated by >1 SD from baseline was $T_1 = -8$ ms and $T_2 = 28$ ms, respectively. In the monotic condition, the earliest T_1 time was -8 ms and the longest T_2 time was 28 ms, and both were determined using a change in LSL. In the monotic condition, the latency of inhibition preceded the excitatory FSL by 4.68 ms, and this inhibition persisted 10.68 ms longer than the NE tone. **E**, Dot raster display illustrating responses of the same DTN to dichotic paired tone stimulation (BD tone to contralateral ear; NE tone to ipsilateral ear). The inhibition evoked by the NE tone caused a brief change in the spike count (**F**), FSL (**G**), and LSL (**H**) of the cell. In the dichotic condition, the latency of inhibition lagged the excitatory FSL by 5.09 ms, and the duration of the inhibition was 2.91 ms. This example shows that the inhibition evoked by the NE tone in the dichotic condition was weaker, shorter in duration, and occurred at a longer latency than the inhibition evoked in the monotic condition. Contra, Contralateral; Freq, frequency; Ipsi, ipsilateral; Thresh, threshold.

Figure 3 shows an example of responses evoked from a bandpass DTN tested with monotic and dichotic paired tone stimulation using equal-amplitude BD and NE tones. In contrast to the example shown in Figure 2, spike suppression was observed in

this cell for both the monotic and dichotic conditions. In the monotic condition, when the 2 ms BD tone and the 20 ms NE tone were presented to the contralateral ear (Fig. 3A), again there was nearly complete response suppression as the offset of the BD

tone approached the onset of the NE tone (Fig. 3B) but with no change in FSL when spikes were evoked; however, a clear increase in FSL was observed for spikes that were evoked near the end of the stimulus overlap (Fig. 3C). Note also the change in LSL as the BD tone approached the NE tone (Fig. 3D). This decrease was caused by the inhibition evoked from the stationary NE tone suppressing the last spikes in the responses evoked by the BD tone (Fig. 3A). The final values for T_1 and T_2 were -8 and 28 ms, respectively, and were derived from LSL changes. The effective duration of spike suppression was calculated to be 30.68 ms. This neuron had leading inhibition because $L_{\text{first}} = 11.70$ ms and $T_{\text{start}} = 7.02$ ms, hence the onset of inhibition preceded the FSL by 4.68 ms. The cell also had persistent inhibition because the effective duration of spike suppression lasted 10.68 ms longer than the 20 ms NE tone.

In the dichotic condition, response suppression was again observed when the roving BD tone was presented to the contralateral ear and the stationary NE tone was presented to the ipsilateral ear; however, the inhibition evoked by the NE tone caused only a brief change in the spike count (Fig. 3F), FSL (Fig. 3G), and LSL of the cell (Fig. 3G). The final value for T_1 was 2 ms and was derived from spike latency changes; the final value for T_2 was 10 ms and was derived from a consensus between spike count and LSL changes. The effective duration of spike suppression was 2.91 ms. In contrast to the monotic condition, the suppression observed in the dichotic condition was weaker, shorter in duration, and delayed until after the BD tone had completely overlapped the NE tone. The cell did not show leading inhibition in the dichotic condition because $L_{\text{first}} = 10.98$ ms and $T_{\text{start}} = 16.07$ ms, hence the onset of the inhibition was delayed by 5.09 ms relative to the FSL. The cell did not have persistent inhibition because the effective duration of spike suppression was 17.09 ms shorter than the 20 ms NE tone. The change in the latency, strength, and duration of the response suppression in the dichotic condition suggests that the inhibition evoked through stimulation of the ipsilateral ear differed from the inhibition that was evoked through stimulation of the contralateral ear.

All 42 DTNs exhibited spike suppression in the monotic condition, but only 20 cells (47.6%) showed spike suppression in the dichotic condition with equal-amplitude BD and NE tones. This difference was highly significant (Fisher's exact test, $p < 0.0001$). The finding that less than half of the DTNs showed no evidence of inhibition recruited by stimulation of the ipsilateral ear supports the hypothesis that the inhibition and neural circuits responsible for creating the temporally selective responses of DTNs are monaural.

We measured the baseline FSL (L_{first}) and latency of the inhibition evoked by the NE tone (T_{start}) for each DTN tested with monotic and dichotic paired tone stimulation and calculated the latency difference ($L_{\text{first}} - T_{\text{start}}$) to determine whether the inhibition preceded, was simultaneous with, or followed excitation. A positive difference indicates that inhibition led excitation; a negative difference indicates that inhibition lagged excitation (see above, Materials and Methods, Determining the time course of inhibition). The histograms in Figure 4 show the number of DTNs with leading and lagging inhibition in the monotic and dichotic conditions when the BD and NE tones were equal in amplitude (but not in energy). In the monotic condition (Fig. 4A), leading inhibition was observed in 34 of 42 cells (80.95%); however, in the dichotic condition (Fig. 4B), only 4 of 20 cells (20%) showed leading inhibition. The proportion of DTNs with leading inhibition in the monotic and dichotic conditions was significantly different (Fisher's exact test, $p < 0.0001$). In the monotic condition, the mean $\pm$ SD difference of $L_{\text{first}} - T_{\text{start}}$ was

Figure 4. Histograms of the difference between the excitatory FSL and the latency of the inhibition evoked by the NE tone for DTNs tested with monotic and dichotic paired tone stimulation. Cells with a positive latency difference are illustrated with filled bars and represent neurons in which the onset of inhibition preceded the baseline FSL (i.e., leading inhibition). Cells with a negative latency difference are illustrated with open bars and represent neurons in which the onset of inhibition lagged the baseline FSL (i.e., lagging inhibition). **A**, The majority of DTNs tested in the monotic condition had leading inhibition. **B**, A majority of DTNs tested in the dichotic condition had lagging inhibition. The distributions of $L_{\text{first}} - T_{\text{start}}$ in the monotic and dichotic conditions were significantly different (Mann–Whitney $U = 742.00$, $p \ll 0.001$).

3.55 ± 3.92 ms, whereas in the dichotic condition, it was -1.52 ± 2.91 ms; the difference between the two distributions was statistically significant (Fig. 4). These data reveal that the inhibitory inputs recruited by stimulation of the contralateral ear during monotic stimulation have a shorter latency than the inhibitory inputs recruited by the ipsilateral ear during dichotic stimulation.

The histograms in Figure 5 illustrate the time course of the inhibition evoked during monotic and dichotic paired tone stimulation relative to the duration of the NE tone that evoked the inhibition. The plots show the effective duration of inhibition minus the duration of the NE tone (i.e., $T_{\text{end}} - T_{\text{start}} - D_{\text{NE}}$). A positive difference indicates persistent inhibition; a negative difference indicates that the inhibition evoked by the NE tone was shorter in duration than the NE tone itself (see above, Materials and Methods, Determining the time course of inhibition). We observed persistent inhibition in 42 of 42 DTNs (100%) tested in the monotic condition with equal-amplitude BD and NE tones. In some cells, the inhibition persisted >50 ms after the offset of the NE tone (Fig. 5A). In the dichotic condition, only 20 of 42 neurons (47.6%) were inhibited by the NE tone, and the total duration of this inhibition was usually shorter than the duration of the NE tone that evoked it (Fig. 5B). Indeed, persistent inhibition was observed in only 5 of 20 DTNs (25%) tested in the dichotic condition. The difference in the proportion of cells with persistent inhibition in the monotic and dichotic conditions was highly significant (Fisher's exact test, $p < 0.0001$). In the monotic condition, the mean $\pm$ SD difference of $T_{\text{end}} - T_{\text{start}} - D_{\text{NE}}$ was 19.70 ± 13.22 ms, whereas in the dichotic condition, it was -2.32 ± 17.25 ms; the difference between the two distributions was statistically significant (Fig. 5). These data reveal that the inhibition recruited by the contralateral ear during monotic paired tone stimulation persistent longer than the inhibition recruited by the ipsilateral ear during dichotic paired tone stimulation.

Comparing monotic and dichotic inhibitory responses

To highlight differences in the latency and time course of the inhibition evoked by the NE tone during monotic and dichotic paired tone stimulation, we conducted a detailed paired analysis

Figure 5. Histograms of the difference between the duration of the inhibition evoked by the NE tone and the duration of the NE tone for DTNs tested with monotic and dichotic paired tone stimulation. Cells with a positive latency difference are illustrated with filled bars and represent neurons in which the duration of inhibition was longer than the duration of the NE tone that evoked the inhibition (i.e., persistent inhibition). Cells with a negative latency difference are illustrated with open bars and represent neurons in which the duration of inhibition was shorter than the duration of the NE tone that evoked the inhibition. **A**, Every DTN tested in the monotic condition had persistent inhibition. **B**, The majority of DTNs tested in the dichotic condition had durations of inhibition that were shorter than the duration of the NE tone presented to the ipsilateral ear. The distributions of $T_{\text{end}} - T_{\text{start}} - D_{\text{NE}}$ in the monotic and dichotic conditions were significantly different (Mann–Whitney $U = 787.00$, $p \ll 0.001$).

on the responses of those 20 DTNs with measurable inhibition in both conditions using equal-amplitude BD and NE tones. The latency of the inhibition (T_{start}) evoked during monotic stimulation was significantly shorter than the latency of the inhibition evoked during dichotic stimulation in the same cells (Fig. 6A). Moreover, the effective duration of the inhibition ($T_{\text{end}} - T_{\text{start}}$) evoked in the monotic condition was significantly longer than it was in the dichotic condition in the same cells (Fig. 6B). When we subtracted the latency of the inhibition from the baseline FSL, most DTNs showed leading inhibition in the monotic condition but had lagging inhibition in the dichotic condition (Fig. 6C). We also compared the effective duration of the inhibition relative to the duration of the NE tone that evoked the inhibition ($T_{\text{end}} - T_{\text{start}} - D_{\text{NE}}$) and observed that all DTNs had persistent inhibition in the monotic condition, whereas few had persistent inhibition in the dichotic condition (Fig. 6D). Importantly, these results did not depend on the criteria used to measure the inhibition because all differences remained significant when the suppression was quantified using only changes in spike counts or spike latencies (data not shown). Altogether, these data suggest that a subpopulation of DTNs can receive two distinct types of inhibitory inputs: (1) one that is fast and long-lasting, evoked by stimulation of the contralateral ear, and responsible for creating duration-selective responses; and (2) another that is slower and shorter-lasting, evoked by stimulation of the ipsilateral ear, and participates in the binaural response of the cell.

Relation of leading/lagging inhibition to BD, FSL, and duration filter characteristic

Conceptual and computational models of DTNs predict that the BD and range of duration selectivity will depend, in part, on the amount of time by which inhibition leads excitation (Casseday et al., 1994, 2000; Aubie et al., 2009, 2012). A previous study reported a positive correlation between the amount of leading inhibition and the neuronal BD in a sample of DTNs tested with monotic paired tone stimulation (Faure et al., 2003). That study also reported a positive correlation between leading inhibition and FSL.

We measured the latency of inhibition evoked by the NE tone during monotic and dichotic paired tone stimulation and subtracted this from the baseline FSL of the cell in each condition to evaluate the relationship of leading/lagging inhibition to the BD, FSL and duration filter characteristic (Fig. 7). In the monotic condition, there was a positive correlation between the BD and the time by which contralateral inhibition led excitation (Fig. 7A). Leading inhibition was also significantly larger in bandpass DTNs than short-pass DTNs (bandpass = 4.60 ± 0.94 ms; short-pass = 1.60 ± 0.89 ms; Mann–Whitney $U = 81.00$, $p \ll 0.001$). These findings replicate the results of Faure et al. (2003). In the dichotic condition, there was no relation between the BD and the amount of leading/lagging inhibition (Fig. 7B). Moreover, there was no difference in the amount of leading/lagging ipsilateral inhibition between bandpass and short-pass DTNs (bandpass = -1.92 ± 1.08 ms; short-pass = 1.60 ± 0.89 ms; $t_{(18)} = 0.443$, $p = 0.663$).

In the monotic condition, there was a positive correlation between the amount of leading/lagging inhibition and the baseline FSL; DTNs with short FSLs had shorter durations of leading/lagging inhibition than cells with longer FSLs (Fig. 7C). This finding was also reported by Faure et al. (2003); however, in the present study, the difference in FSL between bandpass and short-pass DTNs was not significant (bandpass = 14.39 ± 1.05 ms; short-pass = 13.45 ± 1.15 ms; Mann–Whitney $U = 174.00$, $p = 0.247$). In the dichotic condition, there was no correlation between leading/lagging inhibition and the baseline FSL (Fig. 7D). There was also no difference in FSL between bandpass and short-pass DTNs (bandpass = 13.74 ± 1.05 ms; short-pass = 13.33 ± 1.39 ms; Mann–Whitney $U = 172.00$, $p = 0.227$). Together, these data support the following hypotheses. First, monaural inhibition from the contralateral ear is responsible for shaping the BD, FSL, and duration filter characteristic of DTNs. Second, inhibition from the ipsilateral ear, when present, is not a major determinant of BD, FSL, or the duration filter characteristic of DTNs.

Effect of increasing NE tone amplitude in the dichotic condition

Finally, we explored the effect of increasing the amplitude of the NE tone during dichotic paired tone stimulation. In this experiment, the amplitude of the BD tone in the contralateral ear was fixed, typically at +10 or +20 dB above threshold, while the amplitude of the NE tone in the ipsilateral ear was varied from +0, +10, or +20 dB relative to the BD tone (Fig. 8A). We limited the dichotic amplitude difference to +20 dB to reduce the likelihood of acoustic crosstalk from the ipsilateral ear suppressing spikes evoked via stimulation of the contralateral ear (Ehrlich et al., 1997).

Figure 8B shows an example of responses evoked from a short-pass DTN as the amplitude of the NE tone was increased in the dichotic condition. This cell did not show ipsilaterally evoked inhibition until the amplitude of the NE tone was 20 dB louder than the BD tone. The duration of response suppression was measured in this cell by a change in spike count, but similar suppressive effects were observed when we examined FSL and LSL changes (latency data not shown). Figure 8C shows an example of responses evoked from a bandpass DTN as the amplitude of the NE tone was increased during dichotic stimulation. This cell did not show ipsilaterally evoked inhibition at any amplitude of the NE tone regardless of the criterion used to measure response suppression (latency data not shown).

Of 21 DTNs tested, only four (19.0%) exhibited spike suppression in the dichotic condition when the BD and NE tones

Figure 6. Comparing the latency and time course of the inhibition evoked in DTNs during monotic and dichotic paired tone stimulation. Only cells that exhibited measurable inhibition in both conditions were included in this paired analysis ($n = 20$). In each panel, the inhibition recruited monaurally is plotted on the x-axis, the inhibition recruited binaurally is plotted on the y-axis, and the dotted line is the unity line ($y = x$) indicating that monaural inhibition = binaural inhibition. **A**, The latency of the inhibition (T_{start}) recruited by the NE tone was significantly longer in the dichotic condition (Wilcoxon's signed-rank test, $Z = -3.845$, $p < 0.001$), as evidenced by the majority of points falling above the unity line. **B**, The duration of the inhibition ($T_{\text{end}} - T_{\text{start}}$) evoked by the NE tone was significantly longer in the monotic condition ($t_{(19)} = 4.605$, $p < 0.001$), as evidenced by the majority of points falling below the unity line. **C**, The difference between the FSL and latency of inhibition ($L_{\text{first}} - T_{\text{start}}$) was usually positive in the monotic condition indicating leading contralateral inhibition but was usually negative in the dichotic condition indicating lagging ipsilateral inhibition (Wilcoxon's signed-rank test, $Z = -3.920$, $p < 0.001$). **D**, The difference between the effective duration of inhibition and the NE tone duration ($T_{\text{end}} - T_{\text{start}} - D_{\text{NE}}$) was always positive in the monotic condition indicating persistent contralateral inhibition but was usually negative in the dichotic condition indicating shorter lasting ipsilateral inhibition ($t_{(19)} = 6.934$, $p < 0.001$).

were equal in amplitude. When the amplitude of the NE tone was increased to +10 dB (relative to BD tone), 9 of 21 cells (42.8%) showed spike suppression. When the amplitude of the NE tone was increased to +20 dB (relative to BD tone), 16 of 21 neurons (76.2%) showed suppression. We also measured the effective duration of the inhibition at each NE tone amplitude; cells that did not meet the criteria for response suppression were assigned a value of 0 ms. A nonparametric equivalent to a repeated-measures ANOVA (Friedman's test) revealed that there was a significant increase in the effective duration of inhibition as the amplitude of the NE tone was increased in the ipsilateral ear (Fig. 8D).

Discussion

Inhibitory inputs that create DTNs are monaural

A number of neural circuit models, backed by electrophysiological studies, have proposed biological mechanisms to explain the formation of duration-selective responses. Although there are variations to these models, most rely on the interaction of excitatory and inhibitory synaptic inputs (Casseday et al., 1994, 2000; Covey et al., 1996; Fuzessery and Hall, 1999; Faure et al., 2003;

Aubie et al., 2009, 2012; Sayegh et al., 2012). One key feature of the models is that DTNs receive a sustained inhibitory input that lasts as long as, or longer than, the duration of the stimulus evoking the inhibition. Combined with limited evidence from whole-cell patch-clamp and single-unit recordings (Casseday et al., 1994, 2000; Covey et al., 1996; Ehrlich et al., 1997; Faure et al., 2003; Leary et al., 2008), the models suggest that DTNs receive inhibitory inputs that precede their excitatory inputs.

Extracellular electrophysiology typically only allows researchers to observe the expression and/or suppression of action potentials. Using methods first described by Faure et al. (2003) and Covey and Faure (2005), we used paired tone stimulation, combined with single-unit recordings, to indirectly measure the latency and duration of the inhibition acting on DTNs. This technique works for DTNs because the paradigm uses an excitatory BD tone that reliably evokes spiking as a probe stimulus and a NE tone that reliably suppresses spikes evoked by the BD tone (Fig. 1). Although we used the NE tone as an inhibitory stimulus, it is important to note that the BD and NE tones activated both excitatory and inhibitory synaptic inputs (Casseday et al., 1994, 2000; Covey et al., 1996; Fuzessery and Hall, 1999; Faure et al., 2003); however, the net effect of the NE tone was inhibitory and the net effect of the BD tone was excitatory (Faure et al., 2003, their Fig. 2B). Therefore, the influence of the NE tone on the spike counts and latencies evoked by BD tone resulted from the combined effects of excitation and inhibition.

In this study, we found that all DTNs exhibited spike suppression during monotic paired tone stimulation when equal-amplitude BD and NE tones were presented monaurally to the contralateral ear, whereas half of the DTNs showed no spike suppression during dichotic paired tone stimulation when the BD tone was presented to the contralateral ear and an NE tone of equal amplitude was presented to the ipsilateral ear (Figs. 2–5). The fact that many DTNs were not inhibited by stimulation of the ipsilateral ear clearly demonstrates that monaural circuits provide the inhibition needed to create duration selectivity. In the monotic condition, the latency of the inhibition evoked by the NE tone was shorter than the FSL of a cell, a finding that is consistent with previous studies showing that the inhibitory inputs to DTNs precede their excitatory inputs. Only half of the DTNs showed spike suppression in the dichotic condition, and of those, most had latencies of inhibition evoked by the NE tone that were longer than the excitatory FSL of the cell, revealing that ipsilateral inhibition usually lagged contralateral excitation (Fig. 6).

In the monotic condition, all DTNs showed sustained inhibition that lasted as long as, or longer than, the duration of the NE

tone, a finding also reported previously (Faure et al., 2003), whereas in the dichotic condition, the duration of inhibition was usually shorter than the duration of the NE tone that evoked the inhibition (Figs. 5, 6). These results indicate that the neural circuits and mechanisms that create duration-selective cells in the auditory midbrain are primarily monaural.

When ipsilateral inhibition was evoked, it was weaker, slower, and shorter in duration than the contralaterally evoked inhibition to the same cell (Fig. 6) but grew with increasing amplitude of the NE tone (Fig. 8). Together, these results demonstrate that the synaptic inhibition that is responsible for creating DTNs in mammals is recruited through monaural, contralateral auditory pathways and is independent of the ipsilateral inhibition that shapes the interaural level difference (ILD) tuning profile of many IC neurons.

Effect of increasing NE tone amplitude in the dichotic condition

The IC receives converging bilateral inputs from a number of monaural and binocular auditory nuclei (Adams, 1979; Zook and Casseday, 1982), so it is natural to expect that the neural inputs responsive to sound stimulation at each ear play a role in shaping the response characteristics of IC neurons. In a previous report on binocular interactions in the IC of the big brown bat, ~61% of cells were classified as EI because they received an excitatory (E) input when the contralateral ear was stimulated and an inhibitory (I) input when the ipsilateral ear was stimulated, and 32% of cells were classified as EO (monaural) because ipsilateral stimulation had no effect (O) on contralaterally evoked responses (Lu and Jen, 2003). In the IC of the cat, the proportion of EI cells ranges from 26 to 41%, depending on stimulus frequency, and the proportion of EO cells varies from 17 to 49% (Semple and Aitkin, 1979; Irvine and Gago, 1990).

In our study, 52% of DTNs showed no evidence of receiving an ipsilaterally evoked inhibition when they were tested with dichotic paired tone stimulation using equal-amplitude BD and NE tones. In some cells, we were able to recruit binocular inhibition by increasing the amplitude of the ipsilateral NE tone relative to the contralateral BD tone (Fig. 8). Increasing the amplitude of the NE tone up to +20 dB (relative to BD tone) induced spike suppression in 12 of 17 neurons that were not suppressed previously when the amplitude of the ipsilateral NE tone was equal to the amplitude of the contralateral BD tone. One possible explanation is that increasing the amplitude of the ipsilateral NE tone induced spike suppression via inadvertent stimulation of the contralateral ear through acoustic crosstalk; however, another study using identical loudspeakers reported >30 dB of attenuation between the ears (Ehrlich et al., 1997), which is well above the +20 dB ILD that we used with our dichotic stimuli. Crosstalk could have evoked an additional inhibitory response and increased spike suppression in the binocular condition when the NE tone was presented at a high amplitude relative to the BD tone, particularly

Figure 7. Relationship of leading/lagging inhibition to BD, FSL, and duration filter characteristic in DTNs tested with monotic and dichotic paired tone stimulation. **A**, The amount of time that inhibition led excitation in the monotic condition increased in DTNs tuned to longer BDs ($r^2 = 0.62$; $p < 0.001$; $n = 42$). **B**, In contrast, in the dichotic condition, there was no relationship between leading/lagging inhibition and BD ($r^2 = 0.01$; $p = 0.67$; $n = 20$). **C**, The amount of time that inhibition led excitation in the monotic condition increased in DTNs with longer baseline FSLs ($r^2 = 0.46$; $p < 0.001$; $n = 42$); **D**, however, there was no relationship between leading/lagging inhibition and baseline FSL for cells tested in the dichotic condition ($r^2 = 0.18$; $p = 0.065$; $n = 20$).

for cells in which the threshold of inhibition was lower than that of excitation. The potential for acoustic crosstalk strengthens our hypothesis that the neural circuitry underlying the formation of DTNs is monaural. Moreover, the fact that some DTNs were never inhibited by stimulation of the ipsilateral ear clearly demonstrates that monaural auditory pathways provide all of the inhibition necessary to create duration-selective responses.

Role of inhibition from the ipsilateral ear

Our results clearly demonstrate that the inhibition responsible for creating the temporally selective responses of DTNs originates from monaural auditory pathways. This inhibition is recruited by stimulating the contralateral ear, is strong, fast, and onset-evoked, precedes excitation to the DTN, and is sustained for as long as, or longer than, the duration of the stimulus. When inhibition was recruited via stimulation of the ipsilateral ear, for the majority of DTNs, it was weaker and longer in latency than either the contralaterally evoked inhibition or excitation (Fig. 6). Our findings raise two important questions for future studies. Why do primarily monaural neurons exist at the level of the auditory midbrain, and what role do DTNs play in normal hearing?

Our results demonstrate that DTNs in the mammalian IC can receive at least two distinct types of inhibitory inputs. The first type is present in every cell, is evoked by stimulation of the contralateral ear, and is responsible for creating the temporally selective responses characteristic of DTNs. The second type of inhibition occurs in a subset of DTNs, is recruited by stimulation of the ipsilateral ear and interacts with the monaural inhibition, and is likely similar to the binocular inhibition evoked in other

Figure 8. Effect of increasing the amplitude of the ipsilateral NE tone during dichotic paired tone stimulation. **A**, Schematic of the stimulus paradigm. A constant-amplitude BD tone was presented to the contralateral ear, and a variable-amplitude NE tone was presented to the ipsilateral ear (decibel increase not to scale). **B**, Short-pass DTN with evidence of ipsilaterally evoked inhibition. Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones when the amplitude of the NE tone was +0 dB (top), +10 dB (middle), and +20 dB (bottom) relative to the BD tone. There was no effect of increasing the amplitude of the ipsilateral stimulus on BD tone evoked responses until the NE tone was +20 dB (relative to BD tone). $D_{BD} = 2$ ms; $D_{NE} = 20$ ms; frequency = 32 kHz; threshold = 32.5 dB SPL; BD tone amplitude = +10 dB relative to threshold; repetitions per stimulus = 20. **C**, Bandpass DTN with no evidence of ipsilaterally evoked inhibition. Mean $\pm$ SE spikes per stimulus as a function of the ISI between the BD and NE tones when the amplitude of the NE tone was +0 dB (top), +10 dB (middle), and +20 dB (bottom) relative to the BD tone. There was no effect of increasing the amplitude of the ipsilateral stimulus on BD tone evoked responses. $D_{BD} = 2$ ms; $D_{NE} = 20$ ms; frequency = 23 kHz; threshold = 33 dB SPL; BD tone amplitude = +10 dB relative to threshold; repetitions per stimulus = 20. **D**, Mean $\pm$ SE duration of inhibition evoked by the ipsilateral NE tone when the amplitude of the NE tone was +0, +10, and +20 dB (relative to BD tone). Increasing the amplitude of the NE tone caused a significant increase in the duration of the inhibition evoked through ipsilateral central auditory pathways (Friedman test, $\chi^2(2, n = 21) = 24.947, p < 0.001$). Contra, Contralateral; Ipsi, ipsilateral.

types of IC neurons. In the IC of bats, DTNs are known to receive two types of inhibitory neurotransmitters, GABA and glycine (Casseday et al., 2000). Although it is tempting to speculate that the two types of inhibitory inputs to DTNs reported in this study are using different neurotransmitters, for now this hypothesis awaits investigation.

Monotic and dichotic temporal masking

The paired tone stimulation paradigm we used on DTNs in the IC of the bat mirrors the design of auditory temporal masking experiments in human psychophysics (Faure et al., 2003). The

change in spike count and latency that occurred when the BD tone preceded the NE tone is the neural equivalent of backward masking and was caused by the leading inhibition evoked by the NE tone suppressing spikes evoked by the BD tone. Similarly, the change in spiking that occurred when the BD and NE tones overlapped in time to form a single composite tone with an amplitude pedestal is the neural equivalent of simultaneous masking and was caused by the sustained inhibition evoked by the NE tone suppressing spikes evoked by the BD tone. The change in spiking that occurred when the BD tone followed the NE tone is the neural equivalent of forward masking and was caused by the

persistent inhibition evoked by the NE tone altering responses evoked by the BD tone. Interestingly, psychophysical studies have shown that forward and backward masking last longer when humans are presented with monotic compared with dichotic stimuli (Elliott, 1962a,b; Deatherage and Evans, 1969). These behavioral findings mirror our single-cell neurophysiological results: DTNs tested with monotic paired tone stimulation had greater amounts of leading, sustained, and persistent inhibition than the same cells tested with dichotic paired tone stimulation (Fig. 6). These results suggest that leading, sustained, and persistent inhibition at the level of the midbrain are neural mechanisms that can help to explain temporal masking phenomena in human psychophysics.

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