Sensitivity to Interaural Time Differences in the Dorsal Nucleus of the Lateral Lemniscus of the Unanesthetized Rabbit: Comparison With Other Structures

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1Department of Neuroscience, University of Connecticut Health Center, Farmington, Connecticut; 2Department of Otalaryngology, University of North Carolina, Chapel Hill, North Carolina; 3Department of Anatomy and Department of Otalaryngology and Communicative Sciences, University of Mississippi Medical Center, Jackson, Mississippi; and 4Connecticut State Department of Public Health, Hartford, Connecticut

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Kuwada, Shigeyuki, Douglas C. Fitzpatrick, Ranjan Batra, and Ernst-Michael Ostapoff. Sensitivity to interaural time differences in the dorsal nucleus of the lateral lemniscus of the unanesthetized rabbit: comparison with other structures. J Neurophysiol 95: 1309–1322, 2006. First published December 7, 2005; doi:10.1152/jn.00901.2005. Interaural time differences, a cue for azimuthal sound location, are first encoded in the superior olivary complex (SOC), and this information is then conveyed to the dorsal nucleus of the lateral lemniscus (DNLL) and inferior colliculus (IC). The DNLL provides a strong inhibitory input to the IC and may serve to transform the coding of interaural time differences (ITDs) in the IC. Consistent with the projections from the SOC, the DNLL and IC had similar distributions of peak- and trough-type neurons, characteristic delays, and best ITDs. The ITD tuning widths of DNLL neurons were intermediate between those of the SOC and IC. Further sharpening is seen in the auditory thalamus, indicating that sharpening mechanisms are not restricted to the midbrain. The proportion of neurons that phase-locked to the tones delivered to each ear progressively decreased from the SOC to the auditory thalamus. The degree of phase-locking for a large majority of DNLL neurons was too weak to support their involvement in processing monaural inputs to generate a sensitivity to ITDs. The response rates of DNLL neurons were on average ~60% greater than in the IC or SOC, indicating that the inhibitory input provided to the IC by the DNLL is robust.

INTRODUCTION

The dorsal nucleus of the lateral lemniscus (DNLL) lies just ventral to the inferior colliculus (IC). Its neurons are grouped into islands that are separated by lemniscal fibers primarily destined for the IC. The DNLL receives ascending inputs from many sources, including the contralateral cochlear nucleus, ipsilateral medial superior olive, and the lateral superior olives of both sides (Glendenning et al. 1981). These inputs largely duplicate those to the central nucleus of the IC and may in fact be collaterals of fibers that also innervate the IC (Shneiderman et al. 1988). Most if not all neurons in the DNLL are GABAergic (Adams and Mugnaini 1984; Shneiderman and Oliver 1989). The major projections of the DNLL are to the DNLL on the opposite side and to the inferior colliculus on both sides (Hutson et al. 1991). The projection to the opposite DNLL and IC appears to be exclusively inhibitory (Chen et al. 1999; Shneiderman and Oliver 1989), whereas the projection to the ipsilateral IC is mixed, i.e., predominantly inhibitory with some excitatory inputs (Shneiderman and Oliver 1989).

Activity of neurons in the DNLL has been shown to alter sensitivity to interaural level differences (a cue for sound location) in IC neurons (Faiagold et al. 1993; Li and Kelly 1992). Neurons of the DNLL itself are sensitive to interaural level differences as well (Brugge et al. 1970; Kelly et al. 1998; Markovitz and Pollak 1994; Yang and Pollak 1994a, b).

The involvement of the DNLL in processing another major cue for sound location, interaural temporal disparities (ITDs), has not been extensively explored. The ITD is the main cue for localizing low-frequency sounds (less than ~2 kHz) along the azimuth and neurons in the DNLL are sensitive to ITDs (Brugge et al. 1970). As part of our continuing investigation of ITD processing of low-frequency sounds at different levels of the auditory system in the unanesthetized rabbit (Batra et al. 1997a,b; Fitzpatrick and Kuwada 2001; Fitzpatrick et al. 2000; Kuwada et al. 1987; Stanford et al. 1992), we report here the response features of ITD-sensitive neurons in the DNLL. Because the DNLL and IC both receive inputs from primary ITD-processing centers (medial and lateral superior olives, MSO and LSO, respectively), one prediction would be that the basic features of ITD sensitivity are similar in both structures. We found solid support for this prediction but also found that consistent with its intermediate anatomical position between the superior olivary complex (SOC) and IC, some ITD properties of DNLL neurons lie between those of the SOC and IC.

METHODS

Single-unit recording was performed in nine female Dutch-Belted rabbits (1.5–2.5 kg). Surgical and experimental procedures have been described previously (e.g., Batra et al. 1989; Kuwada et al. 1987) and will be only briefly outlined here.

Surgical procedures

All surgery was performed using aseptic techniques on rabbits with clean external ears. Under anesthesia (ketamine: 35 mg/kg and xyla-
zine: 5 mg/kg im) a square, brass rod was anchored to the skull using screws and dental acrylic. Several days later, the animal was anesthetized, and a small craniotomy (~3 × 4 mm) was made over the intended structure. The craniotomy was covered with sterilized medical elastopolymer (Smith and Nephew Rolyan). At this time, custom ear molds were made for sound delivery.

**Recording procedures and data collection**

All recordings were conducted in a double-walled, sound-insulated chamber. The rabbit was placed in a body stocking from which its head protruded. It was then seated in a padded cradle and further restrained using nylon straps. The stocking and straps provided only mild restraint, their primary purpose being to discourage movements that might cause injury to the rabbit. The rabbit’s head was fixed by clamping to the surgically implanted rod. Once the rabbit was secured, the craniotomy was exposed. To eliminate possible pain or discomfort during the penetration of the electrode, a topical anesthetic (marcaine) was applied to the dura for ~5 min and then removed by aspiration. With these procedures, rabbits remained still for a period of ~2 h, an important requirement for single-neuron recording. Typically, a rabbit participated in daily recording sessions over a period of 2–6 mo. A session was terminated if the rabbit showed any signs of discomfort. The rabbit’s comfort was a priority both for ethical reasons and because movements made it difficult to record from single neurons.

Action potentials were recorded extracellularly from single neurons with glass-coated, platinum-tungsten microelectrodes (tip diameter of 1–2 μm, impedances of 10–30 MΩ). The recordings were amplified 2,000–20,000 times, and the action potentials from a single neuron were isolated with the aid of a time/amplitude window discriminator (BAK Electronics, Germantown, MD) and timed relative to the stimulus onset with an accuracy of 10 μs.

**Acoustic stimulation**

Stimuli were generated by a two-channel digital stimulation system (Rhode 1976) and delivered independently to the two ears through Beyer DT-48 earphones coupled to the custom-fitted ear molds to form a sealed system. Pure tones were gated on and off with linear rise/fall times of 4.0 ms. In later animals, amplitude and phase of tones (60 Hz to 40 kHz in 20-Hz steps) were calibrated prior to the first recording session in each animal by means of a probe tube that extended ~1 mm from the end of the sound tube. The sound tube extended to within ~2.5 cm of the tympanum. This calibration was used to deliver tones during all the recording sessions. In earlier animals, the sound levels and phases used during the recordings were based on calibrations performed on a brass tube with dimensions similar to that of the rabbit ear canal. The responses were later corrected, based on a calibration done in the ear canal after all recordings on that animal were completed (see Batra et al. 1997a; Kuwada et al. 1987).

Sensitivity to ITDs was primarily assessed using binaural-beat stimuli. This stimulus was created by delivering tones to the two ears that differed by 1 Hz, which resulted in a continuously varying ITD that ranged over plus or minus half a period of the tone (Kuwada et al. 1979). The duration of each binaural beat stimulus was usually 5.1 s delivered every 5.3 s and presented two to three times at each frequency. The frequencies tested initially were those from 300 to 1,500 Hz in 200-Hz steps, but higher and lower frequencies were tested if the neuron displayed ITD sensitivity at the boundaries of this range. Intermediate frequencies were also tested. Almost all neurons were tested at 70 dB SPL to allow proper comparisons between auditory structures. Time permitting, the best frequency of a neuron was estimated by delivering short tone bursts (e.g., 75 ms every 150–300 ms repeated 10 times) from 250 to 35,000 Hz at 50–70 dB SPL in 0.5-octave steps.

**Recording sites**

We estimated the location of recording sites in each animal by stereotaxic measures. For each recording, the location of the electrode was set relative to a benchmark on the skull that in turn was referenced to bregma. The anterior-posterior, medial-lateral, and dorso-ventral coordinates determined in this way were then referenced to electrolytic lesions made in the DNLL at the end of recording in each animal (see Fig. 1). By examining the boundaries of the DNLL and lesions with the other side can be seen adjacent to the DNLL (between arrows). This lower lesion was within bands of labeled fibers and cells that correspond to the darkly staining columns seen with parvalbumin (B). Scale bar for A and C is 1 mm and for B and D is 0.5 mm.
the position of the lesions, we determined by our coordinate system if the neurons were in or near the DNLL. The location of the DNLL could also be identified through physiological criteria (see RESULTS).

Data analysis

CONTRALATERAL, IPSILATERAL, AND INTERAURAL SYNCHRONY. The degree to which a neuron synchronized to the frequencies at each ear and the binaural-beat frequency was assessed using synchronization coefficients (vector strengths) to these three components (Goldberg and Brown 1969; Kuwada et al. 1987; Yin and Kuwada 1983). Significant synchrony to these components was assessed using the Rayleigh test of uniformity \((P < 0.001, Mardia 1972)\). Only responses that were significantly locked to the beat frequency were considered to be ITD sensitive and included in the data set. This assessment was done at 70 dB SPL for almost all of the neurons. We have shown previously that the degree of monaural synchrony during a binaural-beat stimulus is comparable to that during monaural stimulation (Batra et al. 1997b).

PEAK- AND TROUGH-TYPE NEURONS. For many of our analyses, we divided our neurons into peak- and trough-type categories based on their characteristic phase (CP). The CP is defined as the phase-intercept at 0 Hz in plots of mean interaural phase versus stimulus frequency of the response (Kuwada et al. 1987; Yin and Kuwada 1983). Peak-type neurons displayed ITD functions the peaks (maxima) of which aligned at a particular ITD across frequency (CPs between 0.0 and ±0.25 cycles). In contrast, trough-like neurons displayed ITD functions whose troughs (minima) aligned at a particular ITD across frequency (CPs within ±0.25 of ±0.5 cycles). To determine the peak of the delay curves, the highest and lowest values of the ITD function were located, and the difference between these points was taken to be the height of the peak or the depth of the trough. The center of the peak was located by fitting by least squares the upper 50% of the peak with a parabola. The center of the trough was located by fitting the lower 50% of the trough (Kuwada et al. 1987).

WIDTH OF ITD TUNING. The width of ITD tuning for each neuron was measured at each frequency. We measured the width of the ITD functions at a point 50% between the maximum and minimum responses. Responses were sometimes irregular, particularly near the edges of the responsive frequency range, so different measurements of this half-width could be obtained if the rate was tracked starting at the maximum or minimum. To identify such irregularities, the width was measured both ways, and if they differed by >33%, the data were not used, otherwise the two measurements were averaged. The width in time could also be expressed in cycles by dividing by the period of the tone.

To obtain a single measurement of the width encompassing the responses to ITDs across the frequencies to which a neuron was ITD-sensitive, composite curves were constructed (Kuwada et al. 1987; Yin and Kuwada 1983). The composite curve was derived by averaging each neuron’s ITD functions across frequency. The method to measure peak width described in the preceding text for responses to a single frequency was unnecessary because averaging the ITD functions across frequency almost invariably produced a smooth function. So, for the composite curves the peak width was simply measured halfway between the maximum and minimum response.

RESULTS

Our results are based on responses of 137 ITD-sensitive, single neurons recorded in the DNLL from nine rabbits. In the DNLL, as in the IC, a large proportion of neurons were sensitive to ITDs, but this proportion was not systematically examined. Where appropriate, comparisons are made between responses in the DNLL and the IC (305 single neurons from 22 animals), auditory thalamus (158 neurons from 8 animals), and SOC (41 neurons from 6 animals). To make valid comparisons between and among neurons in different structures, ITD sensitivity was assessed using the binaural beat stimulus at the same sound intensity (70 dB SPL).

Recording Sites

The DNLL is readily identified through staining for the calcium-binding protein, parvalbumin. It appears as a very darkly staining nucleus located ventral to the also dark-staining IC (Fig. 1A, asterisk). At higher power (Fig. 1B), the nucleus is divided into small patches of darkly stained cells and neuropil (arrowheads), separated by more lightly stained fibers of the lateral lemniscus. In the recording experiments, the DNLL was identified physiologically. As the electrode traversed the lateral part of the IC in a dorsal-anterior to ventral-posterior direction, there was a progression of best frequencies from low to high. Then the electrode encountered a relatively silent zone (~1 mm) where no single neurons could be isolated. Entry into the DNLL was marked by an abrupt transition into a low-frequency zone where the field potential was strong and ITD sensitive neurons with high spontaneous and discharge rates were encountered.

At the end of the experiments, lesions were made at sites where DNLL neurons were recorded, and, in some cases, tracers were placed to examine the connections of the DNLL. For the case in Fig. 1, C and D, a large injection of biotinylated dextran was placed in the left IC and DNLL. On the right side, an electrolytic lesion (10–20 μA for 20 s) was made where a DNLL neuron was recorded (Fig. 1C, bottom arrowhead), and another lesion was made outside the DNLL as the electrode was withdrawn (upper arrowhead). At higher power (Fig. 1D), labeled crossing fibers from the opposite side (between arrows) enter the DNLL, and the lower lesion is seen to be within bands of labeled fibers and cells that correspond to those in the normal DNLL (as in Fig. 1B). We used both stereotactic coordinates relative to the location of electrolytic lesions and the physiological criteria described in the preceding text to determine if a recording site was within the DNLL. We recognize that the location of each recording site is subject to error in chronic studies because many recordings are made over months in a single animal and marking lesions are only made at the termination of the experiment. However, the characteristic physiological pattern described above gave us confidence that our recordings were from neurons in the DNLL.

DNLL: types of ITD sensitivity

Like ITD-sensitive neurons of the SOC (Batra et al. 1997a), IC (Fitzpatrick et al. 2002; Kuwada et al. 1987), auditory thalamus (Stanford et al. 1992), and auditory cortex (Fitzpatrick et al. 2000) studied in unanesthetized rabbits, such neurons in the DNLL also displayed peak- and trough-type responses (Fig. 2). By our convention, positive delays are delays of the stimulus to the contralateral ear. For peak-type neurons, the ITD functions across frequencies aligned at or near maximal discharge (Fig. 2A), whereas those of trough-type neurons aligned at or near the minimal discharge (Fig. 2B). In addition, many neurons had ITD functions that aligned at an intermediate position (Fig. 2C).

The alignment of the ITD functions was determined by a least-squares, response-weighted, linear fit to the plot of the
mean interaural phase versus stimulating frequency (Fig. 2, right). The slope of this fit is the characteristic delay (CD) and the phase intercept at 0 Hz is the characteristic phase (CP). The CP is a measure of whether the alignment occurred at the peak (CP near 0 cycles) or trough (CP near 0.5 cycles). For neurons with CPs near 0 or +0.5 cycles, the CD is an estimate of the difference in conduction times from each ear to the binaural coincidence detector, i.e., the ITD required for coincident arrival of the inputs at each frequency. For neurons with intermediate CPs, the CD represents the ITD where the response amplitude shows the most constancy across frequency, which occurs at an amplitude between the peak and trough. The best ITD of the neuron was estimated from the peak of a parabolic fit to the peak of its composite curve (Fig. 2, middle column, see METHODS). The best ITD measured from the composite curve is very similar to the ITD of broad band sounds that evokes maximum responses (Batra and Fitzpatrick 2002; Fitzpatrick et al. 2000; Palmer et al. 1990; Yin and Chan 1990; Yin et al. 1986). For peak-type neurons, a close correspondence is expected between a neuron’s CD and its best ITD. However, because the best ITD is based on the peak of the ITD function, it will systematically deviate from the CD as the CP changes from a peak- to a trough-type response according to the formula

$$\text{Best ITD} = \text{CD} + \text{CP} / \text{BF}_{\text{ITD}}$$

where BF_{ITD} is the best frequency for ITD sensitivity, defined as the average frequency obtained when each frequency with significant synchrony to the binaural beat stimulus was weighted by the rate at that frequency.

**Comparisons among the DNLL, IC, and other structures**

**BEST FREQUENCY.** Neurons sensitive to ITDs of low-frequency sounds could be of low or high best frequency. Due to recording time constraints and priorities to assess ITD sensitivity, best frequency was assessed in only a subset of our neurons (DNLL: 45/137; IC: 75/305). Most of these neurons (92%) had best frequencies below 9000 Hz. Figure 3 plots the percentage of neurons in the DNLL and IC that had best frequencies below and above 2,200 Hz. This division was based on the observation that the highest frequency where ITD sensitivity is seen in the rabbit is ∼2,200 Hz. Clearly there were a substantial proportion of ITD-sensitive neurons in the DNLL and IC that were tuned to higher frequencies but were sensitive to ITDs of low-frequency sounds. Furthermore, the proportion of these neurons in the DNLL and IC were similar (34 and 33%, respectively).

CP, CD, AND BEST ITD. A comparison of the distributions of CP, CD, and best ITD between the DNLL and IC is shown in Fig. 4. To simplify our analysis, neurons with intermediate CPs were merged with peak- or trough-type neurons based on their
CP (see METHODS). Grouped this way, peak-type neurons constituted about two-thirds of the population, both in the DNLL (Fig. 4A, 65%) and IC (Fig. 4B, 63%). The distribution of CP in the DNLL did not differ significantly (2-tailed Kolmogorov-Smirnov test, \( P = 1.0 \)) from that in the IC (Fig. 4B).

The distributions of CD in the DNLL and IC were concentrated over the estimated physiological range of ITDs for the rabbit (\( \pm 300 \mu s \)), for both peak- (DNLL: 92%; IC: 83%) and trough-type neurons (DNLL: 94%; IC: 79%; Fig. 4, C and D). More peak-type neurons (DNLL: 87%; IC: 80%) had CDs at ipsilateral delays than trough-type neurons (DNLL: 60%; IC: 60%).

In the DNLL, the CD of trough-type neurons differed significantly from those of peak-type neurons (2-tailed \( t \)-test, \( P < 0.002 \)) and was closer to zero delay. Although the mean CDs for peak- and trough-type neurons in the IC (Fig. 4D) were not significantly different to those in the DNLL (2-tailed \( t \)-test, \( P > 0.15 \)), the distribution in the IC was broader than that for the DNLL (Kolmogorov-Smirnov test, \( P < 0.001 \)). As in the DNLL, trough-type neurons in the IC had smaller CDs than peak-type neurons, but this difference was not statistically significant (2-tailed \( t \)-test, \( P = 0.06 \)).

Like the CD distributions, the mean best ITDs for peak- and trough-type neurons in the IC (Fig. 4F) did not differ significantly from those in the DNLL (Fig. 4E, 2-tailed \( t \)-test, \( P > 0.57 \)). However, the best ITD distribution for peak-type neurons in the IC was broader than those in the DNLL (Kolmogorov-Smirnov test, \( P < 0.001 \)), whereas this distribution for trough-type neurons did not differ significantly between the two structures (2-tailed Kolmogorov-Smirnov test, \( P < 0.274 \)). Unlike the CD, in both structures, the best ITDs of trough-type neurons were widely dispersed with only a few within the physiological range and a weaker bias for ipsilateral delays (see Fig. 4 legend for details). Both of these features of trough-type neurons are expected because such neurons typically have composite delay curves with two peaks of comparable amplitude: one at a large positive ITD and the other at a large negative ITD (e.g., Fig. 2B, middle). The best ITD is that of whichever peak happens to be larger.

In summary, the distributions of CP in both structures were similar, indicating that each structure had the same proportion of peak- and trough-type neurons. The mean CDs of peak- and trough-type neurons were similar in both structures, but the mean best ITD was different between peak- and trough-type neurons. The slightly broader distribution of CD and best ITD in the IC resulted in a slight reduction in the proportions of neurons within the physiological range and biased for ipsilateral delays compared with the DNLL. In both structures, no differences were observed between neurons with high and low best frequencies in the distributions of CD, CP and best ITD. The distributions shown are the pooled samples of neurons including those for which best frequencies (BFs) were not determined.

**POPULATION ITD FUNCTIONS ACROSS FREQUENCIES.** The best ITDs for peak-type neurons in the DNLL and IC were mostly confined to the physiological range of ITDs for the rabbit and biased toward sounds with ipsilateral delays that would be
lateralized to the contralateral side of the head. Because the best ITD is measured from the averaged ITD functions across frequency, we asked if this pattern was also present within the response to a single frequency. One approach to answering this question is to average the ITD functions of peak-type neurons within each frequency in the DNLL and IC. These averaged or population ITD functions in the DNLL (Fig. 5A) and IC (Fig. 5D) all displayed peaks at ipsilateral delays (i.e., those created by sounds in the contralateral sound field) and except for the lowest frequency (300 Hz) appeared aligned at ITDs that were within the estimated physiological range. Moreover, in both structures the frequencies that produced the maximum response were between 700 and 900 Hz.

The best population ITDs (Fig. 5, B and E) in both structures were estimated by fitting a parabola to the peak of the population ITD functions in Fig. 5, A and B. These best population ITDs were relatively constant between 500 and 1,500 Hz (DNLL mean = -155 μs; IC mean = -136 μs), and only at 300 Hz was there a notable increase (DNLL = -277 μs; IC = -322 μs). Another way to examine ITDs at different frequencies is to measure the “peak ITD” for each neuron at each frequency, again by a parabolic fit. Because the ITD function to a single frequency is periodic, for each frequency, the parabolic fit was applied to the peak closest to the neuron’s best ITD. In Fig. 5, C and F, we plot these peak ITDs versus stimulating frequency for peak-type neurons. At each frequency, there was a wide range of peak ITDs and the average of these peak ITDs versus frequency (red line) closely approximated that for the best population ITDs shown in Fig. 5B and E. The range of peak ITDs decreased with frequency approximately according to 1/f (light dashed lines), but most neurons had peak ITDs in the physiological range (horizontal dashed lines).

FREQUENCY RANGES FOR ITD SENSIVITY. We estimated the frequency range for ITD sensitivity in each neuron by determining all frequencies that were significantly locked to the best at 70 dB SPL (see METHODS). The bulk of ITD-sensitivity in the DNLL occurred between 300 and 1,500 Hz with a maximum sensitivity in terms of the proportion of neurons contributing, ~700–900 Hz (Fig. 6A). These features were similar to those in the IC.

The range of frequencies over which individual neurons were sensitive to ITDs was also very similar between the DNLL and IC (Fig. 6B). For both structures, the range was between ~1 and 3 octaves with a mean and modal range of ~2 octaves. The difference between the means was not statistically significant (2-tailed t-test, P > 0.89).
RESPONSE RATE. Neurons in the DNLL discharged at substantially higher rates than those in the SOC, IC, and auditory thalamus. Figure 7A shows the means ± SE of the maximum discharge rates measured from the ITD functions at frequencies between 300 and 1,500 Hz for neurons in DNLL, IC, and auditory thalamus. Data from our SOC sample could only be plotted ≤900 Hz due to the small sample size. Averaging across frequencies, the mean maximum rates in the DNLL were ~65% greater than those in the SOC and IC and ~175% greater than those in the auditory thalamus.

The highest response rates for the DNLL and IC were at 700 and 900 Hz. This is consistent with the population ITD functions that also showed the highest discharge rate for this frequency range (Fig. 5, A and B). Thus the rate measured at the ITD of maximum response in the population ITD function reflected the actual rates of firing rather than the alignment of the ITD functions that were averaged.

Figure 7B plots the maximum response rate averaged across frequency for peak- and trough-type neurons in each structure. Except for the SOC, the average maximum discharge of peak-type neurons is higher than those of trough-type neurons and this difference is significant for the DNLL, IC, and thalamus (2-tailed t-test, P < 0.01).

ITD TUNING WIDTHS. The peak widths of ITD functions of DNLL neurons tended to be intermediate between those of neurons in the SOC and the IC. At almost every frequency, the mean peak width was narrower in the DNLL than in the SOC, and broader than in the IC (Fig. 8A). Peak widths in all these structures were broader than those measured in the auditory thalamus. Thus there is a progressive sharpening of ITD-tuning at higher levels of the auditory system.

To quantify the extent of sharpening at each level, we averaged the widths over the frequency range for which we had data in all four structures (300–1,000 Hz). The decrease in these mean widths was 8% between the SOC and DNLL, 18% between the DNLL and IC, and 34% between the IC and auditory thalamus. The total extent of sharpening between the SOC and auditory thalamus was 50%. All of these decreases were significant (2-tailed t-test, P < 0.05).

Unlike the peak widths measured at single frequencies for both peak- and trough-type neurons (Fig. 8A), for measurement of widths in the composite curves (Fig. 8B), we felt it appropriate to measure the peak widths of peak-type neurons and the trough widths of trough-type neurons. This is because for peak-type neurons, the peaks (maximal response) of the ITD functions tend to align across frequency at a particular ITD. However, for trough-type neurons, the alignment across frequency occurs near the trough (minimal response). So if only peak widths were measured, then it would be expected that the peak widths of the composite curves of peak-type neurons would always be less than the peak widths of the composite curves of trough-type neurons.

The peaks of the composite curves of peak-type neurons systematically narrowed between the SOC and auditory thalamus (Fig. 8B). The widths of these peaks were always narrower than the widths of the troughs of trough-type neurons in the same structure (1-tailed t-test, P < 0.001). In contrast, the widths of the troughs of trough-type neurons remained relatively constant at levels above the SOC, where they were exceptionally broad. The broadness of the troughs in the SOC
was probably because the trough-type neurons in our sample were chiefly sensitive to ITDs at frequencies <1,000 Hz. In higher structures, trough-type neurons were often sensitive to ITDs at and even beyond 1,500 Hz.

**MONOAURAL AND INTERAURAL SYNCHRONY.** Sensitivity to ITDs is created in a binaural neuron by the comparison of the timing of action potentials from the two ears that are synchronized to the waveform of the sounds. The creation of sensitivity to ITDs is believed to occur only in the MSO and LSO. However, the IC also receives a strong input from the contralateral cochlear nucleus and a lesser input from the ipsilateral cochlear nucleus. Although the cells that display high synchrony to low-frequency sounds (viz., bushy cells, octopus cells, and onset choppers) do not project to the midbrain, other cell types that can synchronize, albeit to a lesser degree do so (Rhode and Greenberg 1992). So the potential for sensitivity to ITDs to be created in the DNLL does exist. To examine this issue, we measured the synchrony to the contralateral and ipsilateral frequencies extracted from the response to the binaural beat (see METHODS). Figure 9A plots the percent of neurons in each structure that synchronized to the stimulus at both ears at one or more frequencies at which it was ITD sensitive. In the SOC, ~80% showed such phase-locking, indicating that synchrony to stimuli at each ear is preserved in the output of most SOC neurons. The proportion of such neurons was lower in the DNLL than in the SOC, and declined further in the IC and thalamus.

To determine whether members of the majority of DNLL neurons that synchronize to the frequency at each ear might encode the ITD through coincidence detection, we applied a criterion developed by Batra et al. (1997b). This criterion is based on the premise that at a primary site of binaural interaction the interaural synchronization coefficient will be close to the product of the monaural synchronization coefficients. Thus the predicted ratio of the product of the monaural synchronization coefficients to the interaural synchronization coefficient should be near one if the cell is a primary site for creating ITD sensitivity. If the ratio is much <1, the interaural synchrony is much greater than can be accounted for by its monaural synchrony, and the neuron is unlikely to be involved in primary coincidence detection. Batra et al. (1997b) used a criterion of 0.8 to distinguish primary coincidence detectors.

Figure 9B is a scatter plot of this ratio as a function of stimulating frequency for all frequencies where the synchrony to the frequencies at both ears was significant (P < 0.001, Rayleigh test for uniformity) (Mardia 1972). Most of the synchronized responses in the SOC (69%) had ratios >0.8 (dotted line), whereas only 11% of DNLL and 7% of IC had ratios this large. No responses in the thalamus met this criterion. Furthermore, there was a systematic decrease in this ratio at higher and higher structures along the auditory pathway. Figure 9C summarizes this information by plotting the mean ratios at each frequency for each of the structures.

In summary, although some neurons in all structures display monaural synchrony to both ears, the proportion of such neurons progressively decreases above the SOC. The proportion of responses meeting the coincidence criterion in the SOC is high, indicating that it is a site of primary binaural coincidence detection. In contrast, the proportion in the DNLL and IC is small, indicating that most if not all ITD sensitivity in those structures is inherited from coincidence detection in the SOC.

**DNLL: monaural response patterns**

The response patterns of ITD-sensitive neurons in the DNLL to short, monaural tone bursts (50–100 ms) were quite heterogeneous. Figure 10 illustrates representative examples in the form of post stimulus histograms. Most neurons displayed an excitatory response to stimulation of the contralateral ear. However, this contralateral response could take many forms. The discharge pattern could be robust and sustained (e.g., Fig. 10A–C, F, and G), display a sharp onset transient followed by a low sustained discharge (e.g., Fig. 10D) or a sharp onset transient followed by a sustained suppression (e.g., Fig. 10H). The response to stimulation of the ipsilateral ear also displayed many forms and, in general, was less robust than that to contralateral stimulation. Although the ipsilateral response...
could be robust and sustained (e.g., Fig. 10E), the more common response was an onset discharge only (e.g., Fig. 10C), one followed by a low sustained discharge (e.g., Fig. 10D) or followed by a sustained suppression (e.g., Fig. 10F). Ipsilateral stimulation could also produce only a sustained suppression (e.g., Fig. 10G and H).

Do peak- and trough-type neurons in the DNLL have different monaural signatures? At the site of primary binaural coincidence detection peak-type neurons are thought to be largely excited by inputs from both sides, whereas trough-type neurons are thought to be largely excited by input from one side and inhibited by input from the other side. Neurons in the SOC reflect this dichotomy when inhibition is tested with binaural stimuli (Tollin and Yin 2005); however, this dichotomy is not present for responses to monaural stimulation, especially for the case of trough-type neurons where overt contralateral inhibition is rarely observed (Batra et al. 1997a). Considered as populations, however, peak- and trough-type neurons in the SOC do have different monaural signatures. Peak-type neurons typically exhibit excitation to one ear, with the other ear being unresponsive or excitatory as well (0E, EE, or E0). Trough-type neurons are typically excited by ipsilateral stimulation, and unresponsive or excited by contralateral stimulation (0E or EE). The contralateral excitation may be a consequence of inhibitory rebound. To discover whether this difference persists in the DNLL, we sorted the monaural discharge patterns into four broad categories: EE (contralateral excitatory, ipsilateral excitatory, e.g., Fig. 10, A–F), EI (contralateral excitatory, ipsilateral inhibitory; e.g., Fig. 10, G and H), EO (contralateral excitatory, ipsilateral no response) and an “other” category (II, IO, and IE). As in the SOC, our simple expectations were not met. In the DNLL, the largest proportion of peak-type neurons was EE (Fig. 11A). However, unlike the case in the SOC, the largest proportion of trough-type neurons was EE as well. The second largest category of both peak- and trough-type neurons displayed EI characteristics, whereas in the SOC monaurally evoked inhibition was difficult to demonstrate. Only a few peak- and trough-type neurons were EO despite the substantial number of monaurally unresponsive neurons in the SOC.

In the DNLL, at the neuron’s most favorable frequency, the maximum ITD response was considerably larger than that evoked by monaural tones. For individual neurons, we compared the maximum ITD discharge rate with the sum of the

![FIG. 10. Discharge patterns to monaural tone bursts in DNLL neurons are heterogeneous. A–H: examples of discharge patterns to several presentations of the same tone bursts delivered to the contralateral ear alone and to the ipsilateral ear alone for 8 neurons. Each pair of post stimulus time histograms represents a single neuron’s response to contralateral and ipsilateral tone bursts at or near the frequency that evoked the maximum response to the binaural beat, and all at 70 dB SPL. Frequency of the tone bursts for each neuron is as indicated. Horizontal bars represent the duration of the tone burst.](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.00968.2005/-/DC1/fig10)

![FIG. 11. Peak- and trough-type neurons in the DNLL cannot be reliably identified by their monaural response types. A: plot of the monaural response types for peak (○) and trough-type (■) neurons. The response types were sorted into 4 categories: EE (contralateral excitatory, ipsilateral excitatory), EI (contralateral excitatory, ipsilateral inhibitory), EO (contralateral excitatory, ipsilateral no response), and an “other” category (II, IO, and IE). B: plot of peak- and trough-type neurons as a function of whether they displayed occlusive, summative or facilitative behavior. The response to the optimal ITD was summative if it was within 25% of the sum of the monaural responses, facilitative if it was above this range, and occlusive if it was below this range. The peak discharge rate was calculated as in Fig. 7.](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.00968.2005/-/DC1/fig11)
monaural responses to stimulation of either ear at that frequency (Fig. 11B). We considered the neuron to be summative if its maximal ITD response was within 25% of the sum of its monaural responses, facilitative if it was above this range, and occlusive if it was below this range. Facilitation was most often encountered, both in peak- and trough-type neurons, though the proportion of peak-type neurons was somewhat higher. The smaller number of neurons that displayed summative or occlusive effects could also be either peak- or trough-type.

**DISCUSSION**

Our results demonstrate that ITD-sensitive neurons in the DNLL have many properties that are consistent with its anatomical position along the ascending auditory pathway. Here we will discuss our findings in terms of comparisons to other structures and the functional role of ITD processing in the DNLL.

**ITD response types**

Peak- and trough-type neurons, as well as those displaying intermediate responses, are seen at all levels of the binaural pathway (SOC: Batra et al. 1997a; Moushegian et al. 1975; Spitzer and Semple 1995; Yin and Chan 1990; ventral nucleus of the lateral lemniscus: Batra and Fitzpatrick 1997. IC: Kuwada and Yin 1983; Kuwada et al. 1987; McAlpine et al. 1998; Rose et al. 1966; thalamus: Stanford et al. 1992; cortex: Fitzpatrick et al. 2000; Reale and Brugge 1990). In an early study of the DNLL, Brugge et al. (1970) presented the responses of two ITD cells tested at two or three frequencies. At the time, quantitative techniques for distinguishing peak- and trough-type neurons had not been developed, but one neuron appears to be peak-type and the other possibly trough-type. Our results demonstrate that both peak- and trough-type neurons are present in the DNLL as well as neurons with intermediate characteristics.

The mixture of response types seen in the DNLL is likely to have been inherited, in large part, from the SOC. Peak-type responses have been reported in and around the MSO (Batra et al. 1997a; Spitzer and Semple 1995; Yin and Chan 1990), and trough-type responses have been associated with the LSO (Batra et al. 1997a; Joris 1996). However, both these nuclei seem to contain intermediate-type neurons (i.e., CPs far from 0 or 0.5 cycles). In the MSO, ideal peak-type responses (CP = 0 cycle) are thought to be generated by the convergence of excitatory inputs from each ear. However, MSO neurons also receive inhibitory inputs (Brand et al. 2002; Cant and Hyson 1992). Models involving a contralateral inhibitory input (Batra et al. 1997b; Brand et al. 2002) simulated the intermediate response seen in some neurons in the SOC. Such intermediate responses could also be created by the convergence of inputs from ITD-sensitive neurons in other nuclei (Kuwada et al. 1997a; McAlpine et al. 1998; Oliver and Huerta 1992).

**Characteristic delay and best ITD**

The similarity between the distributions of CDs and best ITDs in the DNLL and IC is probably due to the similar projections to these structures from the MSO and LSO (Brunso-Bechtold et al. 1981; Glendenning and Masterton 1983). The bias of peak-type neurons in the DNLL and IC to have their CDs and best ITDs toward ipsilateral delays (i.e., those created by sounds lateralized to the contralateral side of the head) is likely inherited from the MSO. Neurons in the MSO are also biased toward ipsilateral delays (Batra et al. 1997a; Spitzer and Semple 1995; Yin and Chan 1990) and project to the DNLL and IC on the same side (Brunso-Bechtold et al. 1981; Henkel and Spangler 1983; Glendenning and Masterton 1983). Trough-type neurons in the DNLL and IC showed less bias for CDs at ipsilateral delays. Low-frequency neurons in the LSO are trough-type and slightly biased for delays to the contralateral rather than the ipsilateral ear (Batra et al. 1997a). However, the excitatory projection from the LSO is probably bilateral.

Our data set includes neurons with BFs that span from low frequencies (<500 Hz) to well above the phase locking range (>2,200 Hz, ≈8 kHz in some neurons). We did not observe any differences in the CD, CPs, or best ITDs in neurons with high or low BFs. At the intensity used (70 dB SPL, many fibers with high BF in the auditory nerve show phase-locking to low-frequency sounds. The responses to low-frequency sounds may be derived from these “tails” in the tuning curves, or, alternatively, IC neurons with high BFs may receive inputs from neurons that have low BFs. Supporting a contribution to ITD sensitivity from auditory nerve fibers with high BFs, a psychophysical study showed that subjects with a high-frequency hearing loss discriminated ITDs less well than did the subjects with good hearing across frequencies (Smoski and Trahiotis 1986).

**Population ITD functions**

In the DNLL and IC, the best ITDs and mean peak ITDs across frequencies are biased to ipsilateral delays (i.e., those created by sounds in the contralateral field) and are within the physiological range of the rabbit (~±300 μs) (Heffner and Masterton 1980). They are also roughly constant with stimulus frequency from ~500 to 1,500 Hz. The scatter of peak ITDs increases with decreasing frequency (i.e., follows a 1/frequency function) with many neurons tuned to ITDs that would be created by sounds near the midline. McAlpine et al. (2001) obtained similar results in the guinea pig when the best ITD measured from noise ITD functions was plotted as a function of the neuron’s BF for frequencies >500 Hz. Based on an estimate of ±150 μs for the physiological range for the guinea pig, they concluded that the best ITDs were outside this range. However, a recent study has shown that the physiological range for the guinea pig is approximately ±330 μs for frequencies <1,600 Hz (Sterbing et al. 2003). This indicates that the best ITDs in the guinea pig for neurons with BFs above ~400 Hz occur within its physiological range. A major difference between species is that neurons tuned to very low frequencies (50–250 Hz) appear abundant in the guinea pig (McAlpine et al. 1996), whereas they are relatively rare in the rabbit or cat (Stanford et al. 1992; Yin and Kuwada 1983). At these low frequencies, the best ITDs increase greatly with decreasing frequency as expected from the equation relating best ITD, CD, CP, and BF_{ITD} due to the large number of neurons with CPs other than zero (see Equation, pg. 1312).
Response rates

Neurons in the DNLL had markedly higher discharge rates to the binaural beat stimuli than any other structure we have examined in the auditory pathway of the unanesthetized rabbit. This high discharge rate may be due to an inward rectifying current ($I_h$) that serves to increase the rate of spike repolarization in DNLL neurons (Fu et al. 1997). At first glance, it might be expected that this high discharge rate would be suppressed by the inhibitory projection from the opposite DNLL. However, the opposite DNLL is tuned to ITDs in the opposite hemifield. Thus the overlap of the ITD functions from each side would primarily occur in the region of ITDs around zero ITD and have little, if any, effect on the peak discharge rate that occurs at larger ITDs (Kuwada et al. 1997a). Although factors such as synaptic connectivity and efficiency must also play a role, the high discharge rate of DNLL neurons and their large size, indicative of abundant projections, suggest that the DNLL can have a profound effect on the responses in the IC despite their relatively small number (Henkel et al. 2003; Kulesza et al. 2002).

The maximal discharge rates of peak-type neurons were always higher than those of trough-type neurons in the DNLL, IC and thalamus. This is consistent with the idea that the peak- and trough-type neurons in the DNLL and IC reflect a strong input from the MSO and LSO, respectively. Neurons in the MSO show facilitation in that their peak discharge is higher than the sum of the monaural responses (Goldberg and Brown 1969; Yin and Chan 1990), whereas those in the LSO are summative in that their maximum discharge rate is limited by the discharge rate of the ipsilateral, excitatory inputs (Joris and Yin 1995).

**ITD tuning widths**

We have demonstrated previously that the ITD tuning widths are narrower between the SOC and IC and narrower still between the IC and thalamus (Fitzpatrick et al. 1997; Stanford et al. 1992). Here we have shown that the ITD tuning widths of DNLL neurons sit between those of the SOC and IC (Fig. 8); this is consistent with the location of the DNLL in the ascending auditory pathway. Mechanisms of sharpening the inputs from the SOC may depend on the complex circuitry received by IC and DNLL neurons. Circuits that may contribute to the sharpening are outlined for a peak-type neuron in Fig. 12D. The major excitatory input conferring peak-type sensitivity to the DNLL and IC is from the MSO and inhibitory inputs tuned to ITDs come from the contralateral DNLL and the ipsilateral LSO. For the IC, another inhibitory input is from the ipsilateral DNLL (Fig. 12C). An input from the contralateral DNLL (Fig. 12A) would provide inhibition at ITDs corresponding to sounds emanating from the sound field opposite that from which recordings were made. Overlap between the ITD tuning from the MSO and this DNLL input would primarily be around zero ITD and would thus serve to sharpen the medial slope and diminish the overall width. Neurons with sharpened medial slopes are common in the IC and thalamus (Stanford et al. 1992) and were also observed in our DNLL population. A symmetrical sharpening could arise through inhibitory inputs from trough-type neurons in the ipsilateral LSO (Fig. 12B) that do show some overlap in the IC with those of MSO inputs in the IC (Loftus et al. 2004). In the IC, sharpening could also occur through inhibition produced by a peak-type input from the ipsilateral DNLL (Fig. 12C). In this case, the overall response would drop so that the range of ITDs that could excite the neuron would be reduced.

Despite the many similarities between the DNLL and IC, the tuning to ITDs in the IC was noticeably narrower than that in the DNLL. The additional narrowing may be caused by projections that the IC receives but the DNLL does not. A projection from the contralateral IC appears to be predominantly excitatory, but if it acts through inhibitory interneurons, it could serve to suppress the medial slope of peak-type neurons in the IC, much like the mechanism proposed for the contralateral DNLL (Fig. 12A). In addition, the increased sharpening in the IC may be due to intrinsic projections within the IC on the same side. Most, if not all IC neurons display axon collaterals and many neurons in the IC stain positively for GABA (Oliver et al. 1991).

The trough widths of trough-type neurons were not progressively sharper at higher levels of the auditory pathway. Moreover, they were extremely broad and could exceed the physi-
ological range of the rabbit. Such broad troughs are unlikely to play a role in sound localization because there is little modulation of the discharge rate within the physiological range. This conclusion was also reached by Joris (1996) for trough-type neurons in the LSO sensitive to ITDs in envelopes. A proposed role for trough-type neurons was offered by Fitzpatrick et al. (2000), who noted that the peaks of peak type and peaks of trough-type neurons formed a continuous ITD axis. The ITDs of peak-type neurons fell within the physiological range and therefore may serve a role in sound localization, whereas the peaks of trough-type neurons occurred outside the physiological range and could be used for determining the spaciousness of the acoustic environment (Batra et al. 1997a).

**Phase-locking in DNLL neurons**

The ability to synchronize with high fidelity to the waveform of low-frequency sounds, particularly at the upper frequency limits for phase-locking seen in the auditory nerve, may be an intrinsic membrane property. It is seen in spherical and globular bushy of the cochlear nucleus (Rhode and Greenberg 1992; Rhode et al. 1983), principal cells of the medial nucleus of the trapezoid body (Smith et al. 1998), and principal cells of the MSO (Yin and Chan 1990). All of these cell types have a rectifying membrane characteristic and produce an onset spike or two to depolarizing current (Banks and Smith 1992; Manis and Marx 1991; Smith 1995; Wu and Oertel 1984). Such neurons are rare in the DNLL and IC (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001; Wu and Kelly 1995). Consistent with these membrane properties, synchronization to low-frequency sounds in DNLL neurons rarely extended to the highest frequencies for phase-locking. However, more DNLL neurons phase-locked to some frequencies and often with higher synchronization than was the case for IC neurons. The neurons in these two structures receive similar inputs from the SOC, so the higher phase-locking in the DNLL is presumably due to faster membrane properties or to a lower level of synaptic interaction. Although monaural phase-locking was stronger in the DNLL than in the IC, for the bulk of the neurons, it was not strong enough to qualify them as primary coincidence detectors.

**DNLL: monaural response patterns**

The wide variety of discharge patterns to monaural tone bursts in ITD-sensitive neurons in the DNLL is consistent with earlier reports (Aitkin et al. 1970; Brugge et al. 1970). Such heterogeneity has also been noted in the IC (Kuwada et al. 1984, 1989), and intracellular recordings show both excitatory and inhibitory inputs to monaural stimulation (Covey et al. 1996; Kuwada et al. 1997b).

There was a poor correlation between the polarity of the responses at each ear (e.g., excitatory-excitatory, and excitatory-inhibitory) and ITD response type (peak or trough). There are several reasons for this poor relationship. First, the response types as revealed by monaural stimulation of low-frequency, peak-type neurons presumed to be in the MSO are not exclusively EE and those of low-frequency trough-type neurons presumed to be in the LSO are rarely IE or EI (Batra et al. 1997a; Finlayson and Caspary 1989). Furthermore, an EI input may produce an EO response in the DNLL. Second, there is physiological evidence for convergence of peak- and trough-type inputs in the IC (Batra et al. 1993; McAlpine et al. 1998) and anatomical evidence that the low-frequency, ipsilateral projection from LSO and MSO converge in the IC (Loftus et al. 2004). If a similar convergence occurs in the DNLL, then these neurons may show EE, EI, or EO response patterns. Finally, the inhibition seen to ipsilateral stimulation in the DNLL may arise from inhibitory inputs from the ipsilateral LSO. This combined with monaural inputs from both ventral cochlear nuclei (Shneiderman et al. 1988) and ventral nucleus of the lateral lemniscus (Glendenning et al. 1981) could alter the excitatory and inhibitory monaural responses from the MSO and LSO.

**Summary**

We have identified properties of DNLL neurons that are similar to those of IC neurons, and others that are dissimilar. The similarities include the distribution of parameters of ITD sensitivity that are likely to be inherited through common inputs to the two structures, such as the frequency tuning, distribution of ITDs as a function of frequency (CPs and CDs), and common effects of these parameters on the distribution of best ITDs. Major dissimilarities include increased overall rates and ability to synchronize to tones at each ear in DNLL neurons that may be related to differences in intrinsic cellular properties between DNLL and IC neurons. An additional difference, the intermediate degree of sharpening in ITD tuning in DNLL neurons, may depend on specific circuits carrying ITD information.

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