Binaural Response Properties of Low-Frequency Neurons in the Gerbil Dorsal Nucleus of the Lateral Lemniscus

Ida Siveke,1,2 Michael Pecka,1,2 Armin H. Seidl,2 Sylvie Baudoux,2 and Benedikt Grothe1,2

1Division of Neurobiology, Department Biology II, Ludwig-Maximilians-University of Munich; and 2Max Planck Institute of Neurobiology, Martinsried, Germany

Submitted 6 June 2005; accepted in final form 22 March 2006

Siveke, Ida, Michael Pecka, Armin H. Seidl, Sylvie Baudoux, and Benedikt Grothe. Binaural response properties of low-frequency neurons in the gerbil dorsal nucleus of the lateral lemniscus. J Neurophysiol 96: 1425–1440, 2006. First published April 5, 2005; doi:10.1152/jn.00713.2005. Differences in intensity and arrival time of sounds at the two ears, interaural intensity and time differences (IID, ITD), are the chief cues for sound localization. Both cues are initially processed in the superior olivary complex (SOC), which projects to the dorsal nucleus of the lateral lemniscus (DNLL) and the auditory midbrain. Here we present basic response properties of low-frequency (<2 kHz) DNLL neurons and their binaural sensitivity to ITDs and IIDs in the anesthetized gerbil. We found many neurons showing binaural properties similar to those reported for SOC neurons. IID-properties were similar to that of the contralateral lateral superior olive (LSO). A majority of cells had an ITD sensitivity resembling that of either the ipsilateral medial superior olive (MSO) or the contralateral LSO. A smaller number of cells displayed intermediate types of ITD sensitivity. In neurons with MSO-like response ITDs that evoked maximal discharges were mostly outside of the range of ITDs the gerbil naturally experiences. The maxima of the first derivative of their ITD-functions (steepest slope), however, were well within the physiological range of ITDs. This finding is consistent with the concept of a population rather than a place code for ITDs. Moreover, we describe several other binaural properties as well as physiological and anatomical evidence for a small but significant input from the contralateral MSO. The large number of ITD-sensitive low-frequency neurons implicates a substantial role for the DNLL in ITD processing and promotes this nucleus as a suitable model for further studies on ITD-coding.

INTRODUCTION

Interaural disparities in time and intensity are the cues that animals use to localize sounds in the horizontal plane. Interaural intensity disparities (IIDs) are produced by a wavelength-dependent shadowing effect of the head that is more prominent for high- than for low-frequency sounds. In mammals, IIDs are initially processed by neurons in the lateral superior olive (LSO) via a subtraction mechanism based on excitatory inputs from the ipsilateral ear and inhibitory inputs from the contralateral ear (IE) (Boudreau and Tsuchitani 1968; Yin 2002). Most LSO cells are tuned to high frequencies. Because low frequencies do not create significant IIDs, interaural time differences (ITDs) are the dominant cue for localizing low-frequency sounds (Rayleigh 1907; Thompson 1982). ITDs are first processed in the medial superior olive (MSO), which receives both excitatory and inhibitory binaural inputs. The response of MSO neurons is dominated by a coincidence of the net excitation of the inputs from the two ears (for review: Irvine 1992; Yin 2002). Additionally, it has been speculated that low-frequency LSO neurons might contribute to ITD processing (Joris and Yin 1995). A recent study confirms such an IE-based ITD sensitivity for a small number of neurons in the low-frequency limb of the cat LSO (Tollin and Yin 2005). Data from low-frequency MSO and LSO neurons are sparse because it is notoriously difficult to record from these cells in vivo. Accordingly, the few neurophysiological studies of low-frequency MSO and LSO neurons provide small sample sizes compared with studies dealing with other auditory nuclei (Batra et al. 1997b; Brand et al. 2002; Goldberg and Brown 1969; Spitzer and Semple 1995; Tollin and Yin 2005; Yin and Chan 1990). Therefore most data about ITD-processing and the neuronal representation of ITDs stems from the auditory midbrain, the inferior colliculus (IC), a direct target of the MSO and LSO projections (Caird and Klinke 1987; Kuwada and Yin 1983; McAlpine et al. 1998, 2001; Rose et al. 1966; Yin and Kuwada 1983a,b). Unfortunately, a high degree of convergence of both excitatory and inhibitory projections from numerous lower auditory nuclei, from the opposite IC and from intrinsic connections complicates the interpretation of data derived from the IC (for review Oliver and Huerta 1992). Therefore large data sets are necessary to perform reliable population statistics on IC recordings (D’Angelo et al. 2005; Fitzpatrick and Kuwada 2001; Kidd and Kelly 1996; McAlpine and Palmer 2002; McAlpine et al. 2001). In vivo recordings from IC (e.g., McAlpine et al. 1998) as well as theoretical considerations (e.g., Cai et al. 1998a,b) indicate that the convergence of only two MSO inputs, for instance, could create ITD-properties in the IC that are much more complicated than the ITD sensitivity at the level of the MSO itself.

However, the MSO and the LSO also send strong projections to the dorsal nucleus of the lateral lemniscus (DNLL) (Glendenning et al. 1981; Oliver 2000; Shneiderman et al. 1988), a hindbrain structure ventral of the IC. This nucleus is easier to record single neuron responses from than MSO and LSO and shows more linear and, hence, predictable response properties than IC neurons, at least for high-frequency neurons (Xie et al. 2005). DNLL neurons are known to be sensitive to both IIDs and ITDs (Brugge et al. 1970; Fitzpatrick and Kuwada 2001; Kelly et al. 1998; Kuwada et al. 2005; Markovitz and Pollak 1994). Nevertheless, only a little is
known about the role of the DNLL in low-frequency sound processing.

Here we show that many low-frequency DNLL neurons display response properties strikingly similar to those seen in the superior olivary complex (SOC). However, we also found that a substantial portion of our neurons have response features that are more similar to the properties seen in the IC rather than the SOC.

Methods

Experimental animals

Auditory responses from single neurons were recorded from 74 Mongolian gerbils (Meriones unguiculatus) of both sexes. Mongolian gerbils have a well developed low-frequency hearing and can use ITDs and IIDs for sound localization (Heffner and Heffner 1988; Ryan 1976). Animals used for the experiments were 2–3 m of age. All experiments were approved according to the German Tierschutzgesetz (AZ 211-2531-40/01 + AZ 211-2531-68/03).

Surgical procedures

Before surgery, animals were anesthetized by an initial intraperitoneal injection (0.5 ml/100 g body wt) of a physiological NaCl solution containing ketamine (20%) and rompun (2%). During surgery and recordings, a dose of 0.05 ml of the same mixture was applied subcutaneously every 30 min. Constant body temperature (37–39°C) (Field and Siebold 1999) was maintained using a thermostatically controlled heating blanket.

Skin and tissue covering the upper part of the skull was cut and carefully pushed aside laterally, and a small metal rod was mounted on the frontal part of the skull using UV-sensitive dental-restorative material (Charisma, Heraeus Kulzer). The rod was used to reproducibly secure the head of the animal in a stereotactic device during recordings. Custom-made ear-phone holders were attached to the gerbil head close to the acoustic meatus to form a sealed pressure field sound-delivery system allowing the insertion of ear phones and probe-tube microphones. The animal was then transferred to a sound-attenuated chamber and mounted in a custom-made stereotactic instrument (Schuller et al. 1986). The animal’s position in the recording chamber was standardized by stereotactic landmarks on the surface of the skull (intersections of the bregmoid and lambdoid sutures with the sagittal suture in horizontal alignment) (Loskota et al. 1974). For electrode penetrations to the DNLL, a small hole was cut into the skull extending 1.3–2.6 mm lateral from the midline and 0.5–0.8 mm caudal of the interaural axis. Micromanipulators were used to position the recording electrode according to landmarks on the brain surface, and a reference point was used for all penetrations. The dura mater overlying the cortex was removed carefully, and during the recording session, Ringer solution was applied to the opening to prevent dehydration of the brain. For some recordings, the recording electrode was tilted 10° or 5° laterally.

Typical recording periods lasted 10–14 h. After recordings, the animal was killed without awakening by an injection of 0.1 ml of T61 (BGA-Reg No. T331, Intervet), and the last electrode position was marked by a current-induced lesion (5 mA for 5 s after T61 had been applied) using metal electrodes (5 MΩ). Afterward the head was fixed in 4% paraformaldehyde for 2 days. The brain was removed and placed in 30% sucrose at 4°C for 2 days. The brains were embedded in tissue-freezing medium (Jung, Leica Instruments GmbH, Germany), frozen solid, and mounted in a standard plane for sections. Transverse sections were cut at 45 μm in a cryostat at −21°C. Sections were Nissl-stained, and the recording sites verified using standard light microscopy.

Neuronal recordings

Single-unit responses were recorded extracellularly using tungsten electrodes (1 or 5 MΩ; World Precision Instruments) or glass electrodes filled with 1 M NaCl (~10 MΩ). We did not detect any differences between recordings using either type of electrodes in terms of the recording quality (spike-to-noise ratio, possibility of holding the cells and number of cells recorded per penetration) or neuronal response properties (discharge properties, best frequencies, thresholds, aurality, ITD or IID sensitivity). The recording electrode was advanced under remote control, using a motorized micromanipulator (Digimatic, Mitutoyo, Neuss, Germany) and a piezodrive (Inchworm controller 8200, EXFO Burleigh Products Group). Extracellular action potentials were recorded via an electrometer (npi electronics, Germany or Electro 705, World Precision Instruments), a noise eliminator (Humbug, Quest Scientific) removing residual line noise picked up by electrode, a band-pass filter (VBF3, Kemo) and an additional amplifier (Toellner 7607) and fed into the computer via an A/D converter (RP2-1, TDT). Clear isolation of action potentials from a single cell (signal-to-noise ratio > 5) was guaranteed by visual inspection on a spike-triggered oscilloscope (stable shape and amplitude of the action potential) and by off-line spike cluster analysis based on stable amplitudes of the positive and negative peaks (volt) and stable spikes waveform (Brainware, Jan Schnupp, TDT) (see insets Fig. 5 and 7).

Stimulus presentation and recording protocols

Stimuli were generated at 50-kHz sampling rate by TDT System II or III (Tucker Davis Technologies). Digitally generated stimuli were converted to analog signals (DA-3/2/RP2-1, TDT), attenuated (PA5, TDT) and delivered to the ear phones (Sony, Stereo Dynamic Earphones, MDR-EX70LP). The sound field inside the sealed system was controlled using calibrated probe tube microphones (FG 3452, Knowles Electronics). The microphone signal was amplified (RP2-1, TDT) and transferred to the computer for off-line analysis. The difference of the sound pressure level between the two headphones was <5dB in the range of 100–2,000 Hz and the phase difference was <0.01 cycles.

The standard setting was stimulus duration of 200 ms plus squared-cosine rise/fall times of 5 ms, presented at a repetition rate of 2 Hz. For all recordings, stimulus presentation was randomized. To search for acoustically evoked responses, noise stimuli without interaural time and intensity differences were delivered binaurally. When a neuron was encountered, we first determined its best frequency (BF) and absolute threshold using binaurally identical (IID/ITD = 0) sinus tone stimulation. The frequency that elicited responses at the lowest sound intensity was defined as BF, the lowest sound intensity evoking a noticeable response at BF as threshold. These properties were determined on-line by audio-visual inspection in all neurons and, in almost all neurons (229/254), confirmed by a careful off-line analysis of the frequency versus level response areas. These parameters were used to set stimulus parameters subsequently controlled by the computer. In addition, monaural pure tones and binaural pure tones were presented so that the binaural properties (aurality) could be determined.

Sensitivity to ITDs was primarily assessed by presenting a matrix of pure-tone stimuli with varying ITDs and stimulus frequencies 20 dB above threshold. We presented different ITDs over a range equivalent to at least a cycle of the stimulus frequency (step size 100 or 62,500/0.6/BF μs). ITD sensitivity was tested for between three and nine frequencies around BF. ITDs with the contralateral stimulus leading were defined as positive, ITDs with the ipsilateral stimulus leading as negative ITDs. ITD sensitivity was tested setting the IID to 0 dB.

A subpopulation of the binaurally excitable (EE) low-frequency cells was tested with very short downward-frequency-modulated
sweeps (‘chirps’). To record the waveform of the frequency-modulated-downward sweep stimulus (chirp, see Fig. 1), we used a pressure-field \( \frac{1}{2} \)-in. microphone (Type 4192, Brüel and Kjaer) placed \( \sim 5 \) mm in front of the headphone. Headphone and microphone were tightly connected by a plastic tube to mimic the situation at the ear of the animal. The recorded signal was amplified (Calibration amplifier Type 2636, Brüel and Kjaer), digitized (RP2.1, TDT), and stored on a PC. The frequency was modulated linearly from 2,000 to 100 Hz in 3 ms, including squared cosine-function rise and fall times of 0.5 ms. The repetition interval was 2.5 Hz. Although these stimuli generate considerable spectral splatter, we chose them because, unlike clicks, they did not appear to generate a prolonged ringing response; also, most of the stimulus energy is concentrated in the low-frequency band (Fig. 1). The average monaural latencies were assessed for each ear individually by presenting the chirps monaurally. These stimuli evoked either a single discharge or, at most, two discharges with high temporal precision. We could therefore unambiguously determine those discharges that were evoked by the contralateral or the ipsilateral ear, even when the stimuli were presented binaurally, due to the separation by a given ITD. Binaural chirps with varying interaural delays were presented. The stimulation time of the ipsilateral chirp was kept constant and the delay of the contralateral stimulus was varied in steps of 50, 100, or 200 \( \mu \)s. Maximal interaural delays were \( \pm 1 \) or 2 ms. Stimulus amplitudes were adjusted so that cells responded to monaural chirps with one or two action potentials and were then held constant for all further stimulations.

In a subpopulation of EI neurons we assessed IID sensitivity. A combination of different IIDs was presented by holding the intensity on the excitatory ear constant at 20 dB above the binaural (ITD = 0) threshold while varying the intensity on the inhibitory ear in 10-dB steps between 10 dB below and 50 dB above threshold. The resulting IIDs of \( \sim 30 \) dB (negative values mark higher intensities on the inhibitory ear) to +30 dB were presented for five different frequencies centered on BF. The repetition rate was 4 Hz.

**Data analysis**

All quantifications in this study are based on off-line analysis. Spontaneous activity was defined as a firing rate \( \geq 2 \) Hz. For the analysis of the different response patterns, the mean response to binaural stimuli (IID = 0, ITD = 0) at BF and 20 dB above threshold were used. For analyzing the poststimulus time histogram (PSTH), the period histogram, and the inter-spike interval histogram (ISIH) of 184 DNLL neurons, we defined different response patterns. The response pattern was defined as onset (response exclusively during the 1st 50 ms) or sustained (response over the entire duration of the stimulus). Sustained activity was further divided in phase-locked sustained response and non phase-locked sustained response. Neuronal response was classified as phase-locked sustained (s-I) if the vector strength (Goldberg and Brown 1969) was \( \geq 0.3 \) and the \( P < 0.05 \) criterion in the Rayleigh test was fulfilled (Batschelet 1991). Following the description of response pattern of neurons in the cochlear nucleus by Rhode and Greenberg (1992), we divided the nonphase-locked pattern into primary-like (s-p) and tonic sustained (s-t) response patterns. Both patterns did not show regularity in the period histogram or the ISIH. The s-p types were separated from the s-t types by the mean response at the beginning of the response (in the time interval of 12.5–37.5 ms) and the middle of the response (in the time interval of 87.5–112.5 ms). For the s-p types, the response at the beginning was approximately three times larger than the response to the middle portion of the stimulus, whereas the response for the s-t types was about the same in both intervals.

ITD sensitivity was carefully analyzed and quantified for cells that showed \( \geq 50\% \) modulation (reduction of max. spike rate by \( \geq 50\% \)) in their ITD response rate function when tested at BF. For a detailed analysis of ITD functions, we increased our sample size of ITD-sensitive cells by the addition of 105 DNLL cells from earlier, unpublished and published studies (control group) (Seidl and Grothe 2005) using identical equipment and experimental procedures. The quantifications were based on the interaural phase difference (IPD) functions measured with pure tones at different test frequencies (thereby normalizing the cyclic ITD functions for test frequency). The cells mean interaural phase was calculated for each test frequency via a vector analysis following Yin and Kuwada (1983a). Because stimulus phase changes linearly with frequency, neuronal responses can be plotted as best phase versus frequency and the functions can be extrapolated to zero frequency. The phase at which the graphs intersect with the y axis (at 0 Hz) is called the characteristic phase (CP), a value between \(-0.5 \) and \(+0.5 \) cycles. Depending on the calculated CP, different groups of ITD-sensitive neurons can be distinguished. Peak-type neurons have a CP at or around 0 cycles, reflecting coincidence of binaural excitation, which results in an individual best ITD (eliciting the maximal spike rate) independent of test frequency (Yin and Kuwada 1983b). Similar reasoning is applied to trough-type neurons, although trough-type neurons are characterized by the ITD that generates the minimum responses in the ITD functions. The trough in the ITD function is expected when there is coincidence of excitation from one ear and inhibition from the other. Extrapolation of these phase-frequency plots yield characteristic phases at or around \( \pm 0.5 \) cycles, reflecting that maximal responses occur when excitatory and inhibitory inputs are out of coincidence.

We defined peak-type neurons by an absolute CP of \( 0 – 0.125 \) cycles and trough-type neurons by 0.375–0.5 cycles. According to the locations of the peaks and the troughs, we separated these two types into ipsi- or contralateral peak- or trough-type neurons, depending on whether the peak or trough occurred for ipsi- or contralaterally leading sounds. Furthermore we defined two intermediate types: a peak-intermediate type by a absolute CP within 0.125–0.25 cycles and a trough-intermediate type by a absolute CP within 0.25–0.375 cycles. The slope of the linear fit yielded a quantitative measure of the neuron’s characteristic delay (CD) (Rose et al. 1966; Yin and Kuwada 1983b). Phase plots were considered linear if the linear regression component exceeded the 0.005 level of significance using the test of nonlinearity described by Kuwada and colleges (Kuwada et al. 1987). A subgroup of ITD-sensitive neurons (\( n = 81 \)) was tested for the validity of the assumed linearity of our regression lines of the frequency versus best IPD functions. Of this subgroup 74 (93%)
neurons showed a significant linearity (following Kuwada et al. 1987). Furthermore we tested if weighting each data point (best IPD at certain frequency) by the vector strength and the mean response in a similar manner to that described by Kuwada et al. (1987) and Spitzer and Semple (1995) would change the obtained distribution of different types. We could not find any differences. Almost all calculated CPs (71/81, 88%) were not or at most slightly affected and, hence, their classification of ITD-sensitivity was independent of the method used.

To define the point of steepest slope, the ITD-rate function was fitted by a Gaussian (Matlab; The MathWorks) or sigmoid function (Statistica; StatSoft) and the inflection point closest to zero ITD was determined. Fitted ITD functions obtaining an $R^2$-square $<0.7$ were excluded.

Analysis of the responses to binaural chirp stimuli were conducted by defining time slots during which action potentials should occur in response to the ipsilateral or the contralateral stimulus. These time slots (starting point and width) were based on the spike time latencies measured in response to monaural stimulation. The time slots had a width of $\sim 0.25$ ms. Response rates in these time windows were assessed. For responses with more than one action potential per stimulation, only the first action potential was counted. The average spike time, SD, and variance was determined for analysis of the temporal accuracy of a cell’s response.

Neurons were defined as IID sensitive if ipsilateral (inhibitory) stimulation reduced the maximal response elicited by contralateral (excitatory) stimulation by $>50\%$. The IID of maximal inhibition was defined as the smallest IID (lowest intensity at the ipsilateral, inhibitory ear) that caused maximal suppression of the response to the contralateral stimulus. To calculate the maximal inhibition in percent, we used the following formula: $[((\text{maximal response rate} - \text{minimal response rate})/\text{maximal response rate})*100]$. The IID of 50% inhibition was graphically extrapolated from the calculated 50% response rate of the neurons IID function.

**Immunohistochemistry**

Three animals were used for anatomical studies in which neuronal tracers were injected into the DNLL after recording. Two different tracer cocktails were used: a mixture of biotin (10%; Molecular Probes D-1956, NL) and fluorescein-dextran (10%, Molecular Probes D-1820, NL), or tetramethylrhodamine-dextran (10%, Molecular Probes D-1817, NL) dissolved in 0.9% NaCl. Tracers were injected by iontophoresis (6 µA for 6–10 min). Nine to 10 days after the injection, the animals were killed (chloralhydrat, 50 mg/100 g) and perfused transcardially with heparinized 0.9% buffered saline solution for 5 min under deep anesthesia followed by a buffered solution containing 4% paraformaldehyde and 1% glutaraldehyde for 20–30 min. The fixed brain was removed from the skull and placed in 30% sucrose (until it had sunk) for cryoprotection. Transverse sections of 40 µm were prepared in a cryostate (Leica Microsystems CM 3050S, Nussloch).

The histological methods used in this study have been described in detail elsewhere (Malmierca et al. 2002; Oliver et al. 1997). In short, all sections were incubated in 0.05% TritonX100 for 30 min. For visualization of the biotinylated-dextran amine, the avidin-biotin-horseradish peroxidase (ABC Kit, Vector Laboratories) method was used. For permanent staining of the tetramethylrhodamine-dextran, the slices were incubated with anti-tetramethylrhodamine rabbit IgG (Molecular Probes, NL) over night followed by 30 min incubation with biotinylated anti rabbit (Jackson), and avidin-biotin-DAB. Each third section was counter-stained (Nissl) to allow a clear allocation of the labeled cells. Camera lucida drawings were made with the aid of a drawing tube attached to a Leitz microscope (Dialux 20, Leitz, Wetzlar, Germany). Photomicrographs were made with a digital camera (Polaroid). The retrogradely labeled and DAB-stained neurons in the SOC of three animals were counted under the light microscope and pooled for each nucleus (as defined via the Nissl staining).

**RESULTS**

**General response features of DNLL cells**

BFs ranged from 70 Hz to 5.6 kHz, but more than 2/3 of the neurons (185/254) had BFs $<2,000$ Hz; we refer to these as low-frequency neurons. Twenty percent of the low-frequency DNLL neurons we tested were spontaneously active (38/185; 20.5%; rate: $\approx 2$ Hz).

Low-frequency DNLL neurons (BF $<2$ kHz) exhibited five different discharge patterns when tested at ITD $= 0$. A small group of neurons (34/185, 18%) showed onset responses (Fig. 2A). Of these, two-thirds (23/34, 68%) fired one to three spikes per stimulus with an extremely short onset (on; Fig. 2B), whereas 32% (11/34) showed a phasic-on type response (on-ph; Fig. 2C). Most neurons (151/185, 82%) had sustained discharge patterns. About two-thirds of the sustained neurons (101/151, 67%) showed a significant phase-locked response (s-l) to the low-frequency pure tones (Fig. 2D). Non-phase-locked but sustained neurons exhibited either primary-like (s-p; 14/151, 9%) or tonic (t-s) discharge patterns (36/151, 24%). S-t neurons exhibited a nearly constant discharge rate throughout the entire stimulus duration (Fig. 2E), whereas s-p neurons had a stronger response at the beginning of the response period (Fig. 2F).

We tested the distribution of binaural properties using 127 low-frequency DNLL neurons (Fig. 3). Except for a small number of monaural neurons (16/127, 13%) that were excited by the contralateral ear and unaffected by ipsilateral stimulation (EO), most low-frequency DNLL neurons (111/127, 87%) were binaurally sensitive. Most of these binaural sensitive neurons (73/111, 65%) showed evidence for binaural excitation either by responding to monaural stimulation of either ear alone or by exhibiting binaural facilitation. These neurons were classified as excitatory-excitatory (EE). A substantial number of neurons (33/111, 30%) were excited by the contralateral ear and inhibited by stimulation of the ipsilateral ear (EI) and a smaller number of the neurons (4/111, 5%) were excited by ipsilateral and inhibited by contralateral stimulation (IE).

We looked for correlations between temporal response patterns with the binaural response type (Table 1). Interestingly we found that all onset neurons were EE or EO, whereas sustained responding neurons showed all binaural response types. All monaurally inhibited neurons (EI and IE) showed sustained response patterns. EI and IE type neurons showed s-l and s-p response types to an equal extent, whereas the EE and EO types showed more s-l type than s-p type responses. 50% of the binaural neurons exhibited s-p type activity.

**Features of ITD-sensitive neurons**

We evaluated responses to a wide range of ITDs in 189 binaural low-frequency DNLL neurons. ITD-sensitive neurons were divided into two main groups: peak-type neurons and trough-type neurons (as defined in METHODS). Representative ITD-functions of these two types of ITD-sensitive neurons are shown in Figs. 4 and 5. Both example neurons exhibited a sustained phase-locked discharge pattern (Fig. 4, insets) at favorable ITDs (maxima or “peak” of the functions) and a
decreased response rate with only an on-discharge remaining at unfavorable ITDs (minima or “trough” of the function). While the peak-type neuron had a peak response at a common best ITD independent of the test frequency (Fig. 5A), the trough-type neuron showed a trough at a common ITD independent of the test frequency (Fig. 5B). The characteristic phase (CP, see METHODS) of the peak-type neuron was around 0 cycles (Fig. 5A; CP = 0.068 cycles), the CP of the trough-type neuron around –0.5 cycles (Fig. 5B; CP = –0.514 cycles). We found that the large majority of the peak-type neurons was contralateral (70%, 56/80) and that a smaller group was ipsilateral peak-type neurons (30%, 24/80). The trough-type neurons were roughly equally distributed between ipsilateral (44%, 14/32) or contralateral trough-type neurons (56%, 18/32).

The peak-type neurons and trough-type neurons are the most distinct examples of ITD-tuning. Peak-type neurons were the most common type of ITD function and comprised 42% of the sample (80/189). Trough-type neurons were the least common.

FIG. 2. Response types of binaural dorsal nucleus of the lateral lemniscus (DNLL) neurons in response to binaural 200 ms pure tones at best frequency (BF), 20 dB above threshold. A: distribution of response types. B–F: examples of different response types showing discharges as raster plots and phase histograms (insets) averaging the responses on a cycle-by-cycle basis. Stimuli are indicated below the dot raster as black bars. B: example of an on-type response with 1 or 2 action potentials per stimulus presentation (on). BF = 1,300 Hz. C: on-type neuron with multiple spikes at the beginning of the stimulus (on-ph). BF = 800 Hz. D: response of a neuron with a sustained response showing nearly perfect phase-locking as apparent from the phase histogram (inset) and the high vector strength (VS) derived from it (s-I). BF = 200 Hz. E: neuron with a sustained response which was not phase-locked to the stimulus (s-t). BF = 1,300 Hz. F: typical primary-like discharge pattern with a prominent on-component and a weaker ongoing component (s-pl). In this case, the ongoing component was weakly phase-locked. BF = 1,000 Hz.

FIG. 3. Distribution of aural types of low-frequency DNLL neurons. The 1st letter indicates the overall effect of the contralateral, the 2nd letter of the ipsilateral ear. E, excitatory; I, inhibitory; O, no effect. Note that this notation does not distinguished between excitation and facilitation.
and comprised 17% (32/189) of our sample (Fig. 6). A large group of neurons, however, showed an ITD sensitivity between these two extremes; 41% (77/189) of the neurons showed an intermediate type ITD-sensitivity ($0.125 < CP < 0.375$ cycles; Fig. 7A). According to our definition (see METHODS), we found 20% intermediate peak-type neurons ($37/189; 0.125 < CP < 0.25$) and 21% intermediate trough-type neurons ($40/189; 0.25 < CP < 0.375$ cycles; Fig. 8).

Based on qualitative visual inspections, we observed secondary peaks in the ITD functions in 23% (44/189) of our sample. An example neuron is illustrated in Fig. 7B. In some cases, the secondary peak was evoked by one frequency, but in other neurons, it was evoked by all of the tested frequencies. Secondary peaks were not evenly distributed among the groups of ITD-sensitive types. Eleven percent of the contralateral peak-type neurons (6/56) had secondary peaks and were the least likely type to have secondary peaks. Significantly more ($\chi^2; P < 0.001$) secondary peaks were found in ipsilateral peak-type neurons (50%, 12/24).

For a small number of peak-type neurons, we compared the ITD sensitivity of the first spike to the ITD sensitivity of the ongoing component of the response. A typical example is shown in Fig. 8. Comparing the tone-delay functions at BF the ITD sensitivity of the first spike showed the same feature as the ITD sensitivity of the whole response. In 12 of the 16 neurons (75%), we found the first spike to have a similar sensitivity to ITDs as the ongoing component of the response.

We tested for correlations between types of ITD sensitivity three features: the frequency tuning of the neurons (data not shown), the temporal response pattern (Table 2, top part), and the binaural response type (Table 2, bottom part). Note that this subgroup is representative as it has the same distribution of different ITD-sensitive types as the entire sample. We could not find differences in the frequency tuning of the neurons dependent on the type of ITD sensitivity. Also, the temporal response patterns were not specific for any of the groups. Interestingly, the response pattern could change for different ITDs (Fig. 4). The neurons tended to respond to unfavorable ITDs with onset response pattern and to favorable ITDs with sustained response pattern. The response patterns were tested with pure tones at zero ITD, a common standard procedure that obviously leads to a somewhat arbitrary classification for binaural neurons. Around 80% of the peak and intermediate peak-type neurons showed clear signs of binaural excitation. Some intermediate trough- and trough-type neurons were binaurally excited, but the combined number of EI and IE neurons was much higher in these groups.

**Distribution of ITDs across frequency**

The head width of adult gerbils creates ITDs ranging from 0 $\mu$s for sounds emanating straight ahead to $\sim 120 \mu$s for sounds located 90° laterally (Maki and Furukawa 2005). Hence, the maximal ITDs gerbils can experience range from $-120$ to $+120 \mu$s (= "physiological range"). However, the best ITDs of the peak-type neurons when tested at BF were distributed between $+527$ and $-470 \mu$s as shown in Fig. 9. The average interaural phase of the peak-type neurons was 0.13 cycles ($\pm 0.1$ cycles). The majority of peak-type neurons had best ITDs well outside the physiological range of ITDs (Fig. 9A). In contrast to the peaks, the points of steepest slopes of the functions were distributed around 0 ITD and almost all within the physiological relevant range (Fig. 9B). This holds not only for contralateral but also for ipsilateral peak-type neurons, although the latter had a larger variance in their distribution.

**DNLL neurons are sensitive to ITDs evoked with brief chirps**

A model based on the circuitry of the MSO suggested that contralateral preceding inhibition from the medial nucleus of the trapezoid body (MNTB) might shape the ITD functions (Brand et al. 2002). Here we utilized chirp stimuli that could provide some evidence for the existence of a leading contralateral inhibition. The first panel in Fig. 10 shows separate responses evoked by stimulation of each ear at an ITD of $-900 \mu$s (contralateral signal lagging). The shaded areas depict the expected time of response derived from monaural stimulation of the ipsilateral (left $\square$) and contralateral (right $\square$) ear, respectively. The response to contralateral stimulation was influenced by the leading ipsilateral stimulus, as it was slightly delayed compared with the monaural response, whereas the response to the leading ipsilateral stimulus occurs within the expected time frame. When the contralateral signal was lagging by 600 $\mu$s, the contralateral response was almost absent. Note that shortening the lag of the contralateral stimulus led to a remarkable decline in the accuracy of the ipsilaterally evoked response although the response to the leading (ipsi) stimulus still occurred several hundred microseconds before the expected response to the lagging (contra) stimulus. The SD of the latency at 900 $\mu$s ITD was 58.7 $\mu$s and increased to 313.0 $\mu$s at the ITD of 600 $\mu$s for this example neuron. The most significant feature occurred when the contralateral stimulus lagged the ipsilateral signal by 300 $\mu$s (3rd panel). Now the leading inhibition evoked by the contralateral ear suppressed almost all discharges evoked by the ipsilateral signal. When both stimuli were presented simultaneously (4th panel), the cell responded with a single spike, and with re-established excellent temporal accuracy (SD of latency 69.2 $\mu$s, variance 4.8 $\mu$s).

We tested 24 EE cells for contralateral inhibition preceding excitation with these chirp stimuli. In 14 of those neurons (58%), presentation of a contralateral chirp shortly after an ipsilateral chirp changed the response to the ipsilateral stimulation even though the contralateral net excitation always occurred clearly after the ipsilateral one. In 12 of the 14 cells, the influence was seen as a substantial decrease of the spike rate to the ipsilateral chirp ($>50\%$ to maximum spike rate; average decrease of 12 cells was 88.5%) as depicted in Fig. 10.
In two cells, no decrease in the response was detected, but a strong increase in the jitter of the timing of the response was observed (increase of the SD of the average response latency by 66.0%). This effect was furthermore accompanied in these two neurons by a sudden but consistent increase of the average latency. Three cells showed both an increase in latency simultaneously with a decrease in spike rate and one neuron showed an increase in jitter and a decrease in spike rate.

**IID neurons in the DNLL**

Although we were mainly concerned with ITD processing in this study, we also tested 106 cells for sensitivity to IIDs by holding the intensity at the contralateral ear constant and varying the intensity at the ipsilateral ear. Of the 106 cells, 46 (43%) were sensitive to IIDs, which were all of EI aural type. IID-sensitive EI cells had BFs that ranged from 200 to 5,400 Hz (average 2,011.5 ± 1167 Hz). Sixty-one percent
(28/46) of the cells had BF s <2,000 Hz and were therefore in the low-frequency range.

We measured three features of IID functions for 45 EI cells. We determined the maximal inhibition as a percent of the peak response to binaural stimulation, the point of 50% inhibition of the IID function, and the point of maximal inhibition (Fig. 11).

No significant differences could be found in the IID characteristics of low- and high-frequency neurons. Note that our “high-frequency” cells had BFs still in the range of frequencies that, under natural conditions in the free field, would only create significant IIDs in the near field (Maki and Furukawa 2005).

More than three-quarters of the neurons (35/45) showed a reduction in the response rate of 90% or more, and 19 of these units (42%) exhibited a total inhibition of spikes (Fig. 12A). The response of five neurons could not be inhibited by 80% by ipsilateral stimulation. Almost three-quarters of the cells (33/45, 73%) showed 50% points between IIDs of +10 and

FIG. 5. A: Typical peak-type ITD sensitivity in response to different test frequencies (same neuron as in Fig. 3A). Left: neuron exhibited a maximum discharge at an ITD of 350 μs (contralateral stimulus leading, ⌞) independent of test frequency. All other peaks and troughs of the cyclic ITD functions change systematically (according to the length of the cycle) with test frequency. B: trough-type response (same neuron as in Fig. 3B) exhibited a minimal discharge at ~200 μs (contralateral stimulus leading, ⌞), independent of test frequency. The shapes of the action potentials of all spikes recorded for this stimulation are shown as insets. Right: frequency phase plot is shown. For each test frequency, the best interaural phase difference (IPD) was calculated via a vector analysis of the IPD function. The intercept with the y axis gives the characteristic phase (CP). For the neuron shown in A, the CP is close to 0, indicating the peak-type characteristic of the neuron’s ITD sensitivity. In B, the CP around ±0.5 indicates a trough type characteristic (B, right). The slope of the regression line gives the characteristic delay (400 μs for the peak-type neuron and 200 μs for the trough-type neuron in B).

FIG. 6. Distribution of different types of ITD sensitivity found in the population of DNLL cells tested. See text for details.
The peak of the distribution was at slightly negative values as 18 units had 50% points between -5 and -14 dB (Fig. 12B). The average point of 50% inhibition was at -3 dB (±10.8 dB). The point of maximal inhibition for the majority of the neurons (25/45, 55.6%) ranged between -30 and -39 dB (Fig. 12C). Two cells were already inhibited maximally for IIDs > -10 dB.

Anatomy

We injected retrograde tracers bilaterally in the DNLLs of three animals to evaluate the projections of the SOC to the DNLL. After recording from the DNLL, we injected a mixture of biotin and fluorescein-dextran in the left DNLL and tetramethylrhodamine-dextran in the right DNLL (Fig. 13B). The injections on both sides were made in locations that evoked strong activity to low frequencies. Nevertheless, as almost the whole nucleus was filled by the injections, we are not able to comment on any frequency dependence in the staining. We counted the labeled neurons in the SOC of all three animals under the microscope. In all three cases, we found labeled cells in the contralateral DNLL and in various nuclei of the SOC on both sides (Fig. 13, A and B; Table 3). In agreement with previous studies, strong labeling was found in the ipsilateral MSO (174 of 195 labeled MSO neurons). However, we also found a much smaller number of labeled cells in the contralateral MSO (11%, 21/195). Interestingly, there were also a few double-labeled MSO cells (7%, 14/195), suggesting that some MSO cells project bilaterally to the DNLL (Fig. 13C). Large numbers of labeled cells were found in both the contra- and ipsilateral LSOs with about equal numbers of labeled cells in each LSO (contralateral: 44%, 248/641; ipsilateral: 56%, 357/641). Note that we focused on the MSO projections; those sections are shown in Fig. 13 that support the notion that MSO projects bilaterally to the DNLL. Because LSO and MSO only partially overlap in the sections, only a small portion of the LSO is shown (under representing the labeling of LSO cells). As with the MSO (Fig. 13C), some LSO cells were also double labeled (5%, 37/696). Many labeled cells were located in the nuclei of the trapezoid body with the strongest labeling in the ipsilateral MNTB (95%, 380/401), whereas 5% of the labeled cells were found in the contralateral MNTB (21/401). All labeled MNTB neurons were principle neurons. Much weaker labeling was seen in the other nuclei of the trapezoid body. 24 labeled cells were found in the lateral nuclei of the trapezoid
body (LNTB) at both sides, 15 cells in the ipsilateral ventral nucleus of the trapezoid body (VNTB) and 41 cells in the ipsilateral superior paraolivary nucleus (SPN).

**DISCUSSION**

We found the majority of low-frequency ITD-sensitive cells to show either peak- or trough-type characteristics. Peak-type responses were more prevalent than other response types, indicating a dominance of pure MSO input characteristics. Some cells’ responses, however, seemed to reflect pure LSO inputs, and a substantial number of ITD-sensitive cells were neither pure peak-type nor pure trough-type neurons but rather met criteria for intermediate types. Some neurons, mostly trough- and intermediate-type, showed secondary peaks in their ITD-functions, a feature not reported for cells in the SOC.

In 2/3 of the peak-type neurons, maximal responses occurred at ITDs that correspond to sound sources in the contralateral sound field. However, a subpopulation of DNLL neurons preferred ipsilateral sounds, a finding that may correspond to the weaker but nevertheless substantial contralateral MSO input found in the tracer experiments. Best ITDs of most peak-type neurons were evoked at ITDs that gerbils would never experience, at least not as direct irradiation. However, the slopes of their ITD functions were steepest in the physiological range of ITDs.

The abundance of low-frequency cells we found in the DNLL is consistent with the well-developed low-frequency hearing capabilities in the Mongolian gerbil (Ryan 1976). It is, however, important to stress that we actively focused on low-frequency neurons. Normally, the distribution of BFs in the DNLL reflects the entire audiogram of an animal (mustache bat: Markovitz and Pollak 1993; big brown bat: Covey 1993; free-tailed bat: Burger and Pollak 2001; rat: Bajo et al. 1998; cat: Aitkin et al. 1970), and our unpublished results confirm the existence of a large number of high-frequency neurons in the gerbils DNLL (M Pecka and B Saunier-Rebori, unpublished observation).

Most of the binaural neurons we found were binaurally excited. This is consistent with findings in the DNLL of cats and rabbits, which, like gerbils, are well adapted to hear low frequencies (Brugge et al. 1970; Kuwada et al. 2005). In contrast, EI type neurons have been found to dominate in animals that do not hear low frequencies like rats (Bajo et al. 1998; Kelly et al. 1998) and bats (Covey 1993; Markovitz and Pollak 1994).

We found phase-locked sustained discharge patterns to clearly dominate in the gerbil DNLL. These discharge patterns, however, may change depending on the binaural context. Changes of discharge patterns have been suggested to contribute in sound localization (Koch and Grothe 2000; Middlebrooks et al. 1994) but were not systematically investigated in the present study.

**TABLE 2. Correlation between type of ITD sensitivity, response pattern, and aurality**

<table>
<thead>
<tr>
<th>Response pattern/ Aurality/ Type of ITD Sensitivity</th>
<th>Peak Type</th>
<th>Intermediate Peak Type</th>
<th>Intermediate Trough Type</th>
<th>Trough Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>7 (20)</td>
<td>5 (33)</td>
<td>1 (5)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Sustained (phase-locked)</td>
<td>18 (51)</td>
<td>6 (40)</td>
<td>13 (65)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>Sustained (tonic/like)</td>
<td>10 (29)</td>
<td>4 (27)</td>
<td>6 (30)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>EO</td>
<td>2 (6)</td>
<td>3 (20)</td>
<td>2 (10)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>EE</td>
<td>31 (89)</td>
<td>11 (73)</td>
<td>10 (50)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>IE</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>EI</td>
<td>1 (3)</td>
<td>1 (7)</td>
<td>6 (30)</td>
<td>4 (33)</td>
</tr>
</tbody>
</table>

n = 35, 15, 20, and 12 for peak type, intermediate peak type, intermediate trough type, and trough type, respectively. Percentages in parentheses.

The ITD functions of the peak- and trough-type neurons we found in DNLL are similar to the ITD functions reported in the weaker but nevertheless substantial contralateral MSO input found in the tracer experiments. Best ITDs of most peak-type neurons were evoked at ITDs that gerbils would never experience, at least not as direct irradiation. However, the slopes of their ITD functions were steepest in the physiological range of ITDs.

The abundance of low-frequency cells we found in the DNLL is consistent with the well-developed low-frequency hearing capabilities in the Mongolian gerbil (Ryan 1976). It is, however, important to stress that we actively focused on low-frequency neurons. Normally, the distribution of BFs in the DNLL reflects the entire audiogram of an animal (mustache bat: Markovitz and Pollak 1993; big brown bat: Covey 1993; free-tailed bat: Burger and Pollak 2001; rat: Bajo et al. 1998; cat: Aitkin et al. 1970), and our unpublished results confirm the existence of a large number of high-frequency neurons in the gerbils DNLL (M Pecka and B Saunier-Rebori, unpublished observation).

Most of the binaural neurons we found were binaurally excited. This is consistent with findings in the DNLL of cats and rabbits, which, like gerbils, are well adapted to hear low frequencies (Brugge et al. 1970; Kuwada et al. 2005). In contrast, EI type neurons have been found to dominate in animals that do not hear low frequencies like rats (Bajo et al. 1998; Kelly et al. 1998) and bats (Covey 1993; Markovitz and Pollak 1994).

We found phase-locked sustained discharge patterns to clearly dominate in the gerbil DNLL. These discharge patterns, however, may change depending on the binaural context. Changes of discharge patterns have been suggested to contribute in sound localization (Koch and Grothe 2000; Middlebrooks et al. 1994) but were not systematically investigated in the present study.

**Peak- and trough-type DNLL neurons inherit their ITD features from the SOC**

The ITD functions of the peak- and trough-type neurons we found in DNLL are similar to the ITD functions reported in...
previous studies for MSO and LSO neurons. As for MSO cells, the maximal responses (i.e., peaks) in peak-type neurons were evoked at the same ITD across frequency, and thus their phase-frequency plots were linear with a characteristic phase at or around 0.0 cycles (Batra et al. 1997a,b; Brand et al. 2002; Spitzer and Semple 1995; Yin and Chan 1990), indicative of binaural excitation (EE). Similarly, the minimal responses (i.e., troughs) of trough-type neurons were evoked at a common ITD across frequency as has been reported for low-frequency LSO cells and as indicative of an interaction of excitation and inhibition (Batra et al. 1997a,b; Brand et al. 2004; Joris and Yin 1998; Tollin and Yin 2005). The peak responses of DNLL trough-type neurons also had linear phase-frequency plots with a characteristic phase at or around 0.5 cycles. In addition to these features, we observed that in most peak-type neurons the maximal responses were evoked by ITDs generated in the contralateral sound field, which is consistent with the strong projections to the DNLL deriving from the ipsilateral SOC (Glendenning et al. 1981; Oliver 2000; Shneidermann et al. 1988). In a small number of peak-type neurons, the maximal

**FIG. 9.** Peaks and slopes of the ITD functions of peak-type neurons as a function of BF. Values derived from the ITD functions measured at BF. A: best ITDs as a function of the neurons' BF. Note that best ITDs are not independent of BF and that most best ITDs are outside the physiological range of ITDs. B: points of steepest slopes of ITD functions as a function of the neurons' BF. Points of steepest slopes are independent of BF (in contrast to the best ITDs). The majority of points of steepest slopes are close to 0 ITD and well within the physiological range of ITDs. n(out) = number of point outside the physiological relevant range, n(in) = number of point inside the range.

**FIG. 10.** Example neuron (EE) with apparent inhibition from the contralateral side preceding contralaterally driven excitation. Raster plots show the occurrence of single action potentials in response to “chirps.” The stimulus amplitude was set to a level that elicited 1 spike/stimulus when presented monaurally at either ear. Contra delayed by 900 μs, expected response based on monaural stimulation to the ipsilateral (left) or contralateral ear (right). When the contralateral stimulus was delayed by 900 μs, both stimuli elicited spikes. Note that the response to the lagging, contralateral stimulus was already influenced by the leading stimulus in that it was slightly delayed. A decrease of the interaural delay to 600 μs caused the lagging response to the contralateral stimulus to vanish. Also the response to the ipsilateral stimulus became less accurate (higher jitter), although the response to the contralateral stimulus is expected to occur long after the response to the ipsilaterally evoked response. At an interaural delay of 300 μs, the ipsilaterally evoked response was strongly suppressed. Coincidence of the 2 excitatory inputs seems to occur at 0 ITD, resulting in 1 spike/stimulus but with higher accuracy (lower jitter) compared with the monaural responses.
responses were, however, evoked by sounds that would emanate from the ipsilateral side. This finding can be explained by the contralateral projection we found by tracer injections or by the fact that at least a small number of MSO cells has been found to prefer ipsilaterally leading sounds (Batra et al. 1997a; Yin and Chan 1990). Contralateral projections from the MSO, particularly to the IC, are not uncommon, but there is a remarkable species-specific difference concerning their prevalence (review: Grothe 2000). We also found a substantial number of ipsilateral trough-type neurons, responding minimally to a sound in the ipsilateral sound field, consistent with studies of the SOC of rabbits (Batra et al. 1997a,b). This may reflect differences in the latencies of the excitatory and inhibitory inputs to LSO neurons (Irvine et al. 2001; Park et al. 1996). Such latency differences would not only lead to different positions of troughs but also a widespread distribution of the IIDs of maximal inhibition as found for EI DNLL cells in this study and previously shown for LSO neurons (Markovitz and Pollak 1994; Park 1998). All of these features are consistent with the hypothesis that peak- and trough-type neurons in the DNLL inherit their basic ITD properties from the MSO and LSO, respectively. Kuwada et al. (2005) found a similar distribution of ITD properties in the rabbit DNLL with a large number of neurons showing peak-type sensitivity with maximal responses mostly for stimuli generated in the contralateral sound field. They also found a small number of peak-type neurons that would prefer sounds in the ipsilateral sound field. As in our experiments, the peak- and trough-type sensitivity in the rabbit DNLL could not be exclusively explained by the binaural properties of the neurons. Nevertheless, both studies show that most EE neurons show peak-type and most EI neurons trough-type characteristics.

Although many DNLL neurons seem to simply reflect their SOC input, it is important to note that DNLL cells receive not only excitatory inputs from LSO and MSO but also glycinergic inputs from the LSO and GABAergic innervation from the opposite DNLL via the commissure of Probes (for review, Schwartz 1992). In addition, our data confirm the strong glycinergic input from the ipsilateral MNTB earlier shown for cats and rats (Glendenning et al. 1981; Sommer et al. 1993; Spangler et al. 1985). It appears likely that projections from the opposite DNLL or from sources other than the MSO and LSO may affect processing in DNLL, although the nature of this influence requires further study. To date, two interpretations are available. The first by Kuwada and colleagues (Fitzpatrick and Kuwada 2001; Kuwada et al. 2005) suggests that ITD functions are sharpened along the ascending auditory pathway and that first signs of this sharpening are visible in the DNLL. However, their analysis comes from a dataset that does not account for the BF of neurons. As long as ITD functions are simply created by pure EI and EE interactions and as long as these binaural inputs are perfectly matched in terms of their

![FIG. 11. IID-function of a typical EI neuron. The maximal inhibition occurred when the ipsilateral (inhibitory) stimulus was ≥20 dB more intense than the contralateral (excitatory) stimulus. The response was inhibited by −50% when the stimulus level at the inhibitory ear was −3.5 dB more intense (−3.5 dB IID, extrapolated from the IID function). The contralateral stimulus intensity was kept constant (30 dB above threshold) for all values. BF: 2,400 Hz.](http://jn.physiology.org/)

![FIG. 12. Quantification of the interaural intensity difference (IID) functions measured. A: degree of maximal suppression (in percentage). B: IID of maximal suppression. In the majority of cells the response could be reduced by at most 100%, preferable at negative IIDs (ipsilateral stimulus more intense). C: IIDs of 50% inhibition (IID in dB) showing a rather broad distribution.](http://jn.physiology.org/)
frequency tuning, one could see such a sharpening in the population statistic irrespective of BF. There is, however, abundant evidence for inhibitory input to all MSO neurons (review: Grothe 2003), and even the simple EI model for LSO function has become questionable (Kil et al. 1995; Magnusson et al. 2005b). Therefore a quantitative analysis of the ITD width has to account for BF and cannot be performed far from BF where small differences in the frequency tuning of the multiple inputs may account for significant changes in the ITD functions due to different cochlear delays (compare Shamma et al. 1989). Rather, our data suggest that the straightforward processing of ITDs during static tone bursts, in anesthetized animals, is a simple reflection of the processing in the MSO for peak-type neurons and in the LSO for trough-type neurons. Furthermore, our data may suggest a second possible explanation. High-frequency DNLL neurons have been shown to inherit their IID sensitivity from the LSO. What distinguishes them from LSO neurons may not be the response to static stimuli, but the response in a more complex temporal spatial context. Pollak and colleagues (review: Pollak et al. 2003) concluded that DNLL neurons may be involved in echo suppression. Alternatively, they may be involved in processing other dynamic changes in the spatiotemporal domain. Further studies are necessary to elucidate these processes.

**ITD tuning of peak-type neurons in the DNLL**

The maxima of the ITD functions are clearly outside of the physiologically relevant range for direct sound irradiation for the majority of cells tested. It should be noted, however, that they become more complex when reverberations are present. They, via de-correlation, may create larger ITDs. This could be one possible explanation for finding large best ITDs. However, if maximal ITDs matter as such, one would expect the highest density at ITDs that correspond to the highest behavioral resolution, namely around 0 ITD (Hafer et al. 1975; Makous and Middlebrooks 1990). The fact that only few maxima are found around 0 ITD stipulates a different interpretation. The key issue seems to be that, in contrast to the maxima, the slopes of almost all ITD functions cross the midline (ITD = 0). This finding is consistent with studies in the kangaroo rat and gerbil.
TABLE 3. Retrograde labeling of SOC neurons

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
<th>Double</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSO</td>
<td>++ (357)</td>
<td>++ (284)</td>
<td>(+) (37)</td>
</tr>
<tr>
<td>MSO</td>
<td>+ (160)</td>
<td>+ (21)</td>
<td>(+) (14)</td>
</tr>
<tr>
<td>MNTB</td>
<td>++ + (380)</td>
<td>+ (21)</td>
<td>(+) (0)</td>
</tr>
<tr>
<td>VNTB</td>
<td>+ (15)</td>
<td>– (0)</td>
<td>– (0)</td>
</tr>
<tr>
<td>LNTB</td>
<td>+ (13)</td>
<td>+ (11)</td>
<td>– (0)</td>
</tr>
<tr>
<td>SPN</td>
<td>+ (41)</td>
<td>(+) (2)</td>
<td>– (0)</td>
</tr>
</tbody>
</table>

Number of labeled cells in the superior olivary complex (SOC) after injecting biotin/di[4,5-dihalo]adamine-dextran into the right dorsal nucleus of the lateral lemniscus of three Mongolian gerbils. LNTB, lateral nucleus of the trapezoid body; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; SPN, superior parasympathetic nucleus; VNTB, ventral nucleus of the trapezoid body. ++, heavy labeling (>200 cells); +, moderate labeling (>100 cells); +, weak labeling (>10 cells); (+), few cells labeled (<10 cells); −, no labeling. Data pooled from three experiments.

MSO, the IC in guinea pigs and cats (Brand et al. 2002; Crow et al. 1978; Hancock and Delgutte 2004; McAlpine et al. 2001) but appears to be in contrast to the recently published study on the rabbit DNLL that describes most peaks to be within the physiological range (Kuwada et al. 2005). However, Kuwada et al. do not distinguish between best ITDs recorded at BF and those recorded at other stimulus frequencies, and they do not provide any data concerning the slopes of ITD functions for the stimuli that drive a neuron best (BF). Recording in the low-frequency tail of high-frequency neurons (as discussed by Kuwada et al. 2005) might lead to a very different distribution of best ITDs than recording from neurons with low BFs, in particular if BFs and best ITDs are correlated to adjust the slopes of ITD functions to the physiologically relevant range. If the response peaks underlies ITD coding rather than slopes, it would be difficult to interpret the contribution of low-frequency trough-type neurons (also described for the rabbit DNLL) for which best ITDs change as a function of test frequency. Theoretical considerations suggest that a coding strategy with the maximal slopes close to ITD = 0 would be of considerable advantage at least for low frequencies and, in particular, for small animals because it optimizes the change in firing rate in face of small changes in ITD (Harper and McAlpine 2004; Leibold and van Hemmen 2003; Skottum et al. 2001). Common standards for dealing with ITD data will be needed to determine if the differences observed in studies of ITD coding are species specific. The existence of different coding strategies would imply that different principles of neuronal representations of ITDs are used by different groups of animals, whereas similar observations would suggest that all mammals use a common strategy.

This incompleteness of our current knowledge also concerns the role of the inhibitory MSO inputs in ITD processing. There is increasing evidence that the MSO output does not reflect a perfect cross-correlation based on binaural excitation (Batra and Yin 2004). Several anatomical (Cant and Hyson 1992; Clark 1969; Kuwabara and Zook 1992; Perkins 1973) as well as in vivo studies proposed an involvement of inhibitory inputs in ITD coding (Brand et al. 2002; Carney and Yin 1989; Goldberg and Brown 1969; Grothe and Park 1998; Moussegian et al. 1964; Spitzer and Semple 1995). Specific roles of synaptic inhibition have been suggested based on in vitro recordings (Grothe and Sanes 1993, 1994; Magnusson et al. 2005a) and modeling (Batra et al. 1997a; Brand et al. 2002; Han and Colburn 1993; Zhou et al. 2005). The model by Brand et al. (2002) suggests fast contralateral inhibition preceding excitation driven by the same ear that delays the effective contralateral excitatory postsynaptic potentials, to be an important element in the ITD-encoding neuronal circuit in the MSO (Brand et al. 2002; Grothe 2003). However, an alternative model by Zhou and colleagues (2005) assumes a comparatively slow inhibition but still reliably simulates the effects observed during blockade of inhibition in the MSO (Brand et al. 2002). The fact that we found the first spike of the response to be as sensitive to ITDs as the ongoing component speaks for an immediate and fast effect of the inhibition, but direct evidence of a fast preceding inhibition at the MSO is lacking.

Carney and Yin (1989) showed a suppressive effect they called “early inhibition,” which preceded contralateral excitation in ITD-sensitive cells in the IC in response to clicks. The time courses of suppression they found were similar to the effects we obtained in the DNLL by using chirp stimuli. While Carney and Yin hypothesized that preceding inhibition could be generated in the IC, our results show that this inhibition is already present below the midbrain. The fact that both the IC and the DNLL receive prominent inputs from the MSO supports the idea that preceding inhibition is already created at the MSO, the initial site of ITD processing.

One problem in interpreting the time course of the apparent inhibition elicited by broad-band stimuli like clicks or chirps is the possible involvement of cochlear delays. Because of the time course of the traveling wave in the cochlea, high frequencies elicit earlier responses than low frequencies (Ruggiero 1992). Therefore a mismatch in the frequency tuning of MSO inputs could cause a precedence of one of the inputs due to shorter cochlear delays (compare Shimma et al. 1989). The use of downward frequency modulated chirps in the present study might even enhance such effects. However, a comparison of ITD functions measured with pure tones and noise in the MSO itself revealed similar ITD tuning for both stimuli (Yin and Chan 1990), a finding that can hardly be explained by cochlear delays.

Intermediate type ITD sensitivity

It is unclear to what extent intermediate type ITD sensitivity in DNLL is a result of interactions of multiple inputs and to what extent it is imposed by SOC inputs. Spitzer and Semple (1995) as well as Batra and colleagues (1997a,b) found intermediate-type neurons in the SOC of gerbils and rabbits, respectively. They might be a result of convergence of at least three inputs (Batra et al. 1997b). Similarly, intermediate-type neurons have been found at higher stations like the IC (Fitzpatrick and Kuwada 2001; McAlpine et al. 1998; Yin and Kuwada 1983b), but there is evidence that convergence at the level of the IC itself can account for intermediate type ITD sensitivity at least in some neurons (McAlpine et al. 1998). Our present study was not designed to, and therefore cannot distinguish between, the two possible alternatives.

The feature that is likely to be constructed in the DNLL, and not inherited, is the occurrence of secondary peaks that we observed in many ipsilateral peak-type and intermediate peak-and trough-type neurons. A simple explanation for this would

J Neurophysiol • VOL 96 • SEPTEMBER 2006 • www.jn.org
be that these cells received inputs from several cells that had the same BF, but the CD of at least one of the inputs was slightly different from the CD of the other inputs or even at the opposite side (via contralateral MSO inputs) and, therefore caused a second peak in the ITD-function. Such second peaks have not been seen in the ITD functions of SOC neurons and thus are almost surely a property created in the DNLL.

In summary, it appears that pure peak- and trough-type neurons express ITD sensitivity and other properties that are nearly identical to those seen in the MSO and LSO. This suggests that peak- and trough-type neurons in the DNLL may be appropriate substitutes for the SOC, although the connections from other sources suggest the processing may be different in these neurons with stimuli more complex than tones like multiple or dynamic stimuli.

ACKNOWLEDGMENTS

We thank T. Marquardt for help with computer programming and calibration procedures as well as for interesting and stimulating discussions. We thank G. D. Pollak for intense discussions and J. H. C. Casseday, H. Meffin, and M. Burger for critical comments of the manuscript. Many thanks to M. Malmierca for valuable advice concerning double-labeling experiments.

Present addresses: A. H. Seidl, Virginia Merrill Bloedel Hearing Research Center, University of Washington, Box 357923, Seattle, WA 98195; and S. Baudoux, MRC Institute of Hearing Research Nottingham University Section, University Park, Nottingham, NG7 2RD, UK.

GRANTS

This work was supported by the Max Planck Society, German Research Foundation Grants GR 1205/12–1 and GR 1205/11–3, the Deutsche Studienstiftung (stipend for I. Siveke), and European Molecular Biology Organization (stipend for S. Baudoux).

REFERENCES


