Tuning to Interaural Time Difference and Frequency Differs Between the Auditory Arcopallium and the External Nucleus of the Inferior Colliculus

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Vonderschen K, Wagner H. Tuning to interaural time difference and frequency differs between the auditory arcopallium and the external nucleus of the inferior colliculus. J Neurophysiol 101: 2348–2361, 2009. First published March 4, 2009; doi:10.1152/jn.91196.2008. Barn owls process sound-localization information in two parallel pathways, the midbrain and the forebrain pathway. Extracellular recordings of neural responses to auditory stimuli from far advanced stations of these pathways, the auditory arcopallium in the forebrain and the external nucleus of the inferior colliculus in the midbrain, demonstrated that the representations of interaural time difference and frequency in the forebrain pathway differ from those in the midbrain pathway. Specifically, low-frequency representation was conserved in the forebrain pathway, while it was lost in the midbrain pathway. Variation of interaural time difference yielded symmetrical tuning curves in the midbrain pathway. By contrast, the typical forebrain-tuning curve was asymmetric with a steep slope crossing zero time difference and a less-steep slope toward larger contralateral time disparities. Low sound frequencies contributed sensitivity to contralateral leading sounds underlying these asymmetries, whereas high frequencies enhanced the steepness of slopes at small interaural time differences. Furthermore, the peaks of time-disparity tuning curves were wider in the forebrain than in the midbrain. The distribution of the steepest slopes of best interaural time differences in the auditory arcopallium, but not in the external nucleus of the inferior colliculus, was centered at zero time difference. The distribution observed in the auditory arcopallium is reminiscent of the situation observed in small mammals. We speculate that the forebrain representation may serve as a population code supporting fine discrimination of central interaural time differences and coarse indication of laterality of a stimulus for large interaural time differences.

INTRODUCTION

The interaural time difference (ITD) and the interaural level difference (ILD) of a stimulus are major cues for the sound-source location relative to a listener (for a review, see Blaustein 1997). In the barn owl, the ILD is a major cue for elevational sound localization at high frequencies, while the ITD determines the azimuthal component of head turn toward sound sources (Moiseff 1989a,b; Moiseff and Konishi 1981; Olsen et al. 1989; Poganiatz and Wagner 2001; Poganiatz et al. 2001). While these cues may be ambiguous for sound-source position when analyzed in narrow channels of frequencies, characteristic patterns of ITD and ILD across frequency channels unambiguously identify a location in space (Blaustein 1997; Brainard et al. 1992; Keller et al. 1998). In the barn owl, tuning properties to noise stimuli of sensory neurons in higher-order nuclei relate to behaviorally relevant patterns of ITD and ILD arising in different frequency channels (Arthur 2004; Euston and Takahashi 2002; Keller et al. 1998; Saberi et al. 1999; Takahashi and Konishi 1986). Here we compare the tuning properties to ITD, ILD, and frequency in two populations of neurons from sensory nuclei. These nuclei are high-order stations of two parallel streams in the auditory pathway, the auditory arcopallium (AAr) in the forebrain and the external nucleus of the inferior colliculus (ICX) in the midbrain auditory processing pathway of the barn owl. Each pathway is able to mediate sound localization (Knudsen and Knudsen 1996; Knudsen et al. 1993).

Processing of sound-localization cues is well studied in the owl’s midbrain. In the ICX, the convergence of ITD and ILD information from lower centers leads to a mapped representation of the two cues (Knudsen and Konishi 1978a,b; Takahashi et al. 2003). Single neurons in the auditory space map of the ICX are broadly tuned to frequency (Knudsen and Konishi 1978b; Mazer 1998), typically displaying unimodal tuning almost exclusively to best frequencies >2.5 kHz (Wagner et al. 2007). ICX neurons are sensitive to a pattern of specific ILDs across frequency typical of a certain elevational angle of a sound source (Arthur 2004; Euston and Takahashi 2002; Keller et al. 1998). In response to noise stimuli, they display bell-shaped tuning to ILD. Furthermore, ICX neurons respond to a constant value of ITD across frequency channels, a mechanism that removes phase-ambiguous information present in neurons in earlier stations of processing that are tuned to narrow bands of frequency (Takahashi and Konishi 1986; Wagner et al. 1987). As a result, ITD noise tuning curves of ICX neurons display a main peak and smaller side peaks (Mazer 1998; Saberi et al. 1998, 1999; Takahashi and Konishi 1986).

Processing of localization cues in the forebrain pathway is less well studied. The first station, the nucleus ovoidalis (Ov), receives major inputs from the central nucleus of the inferior colliculus (ICC) (Arthur 2005; Cohen et al. 1998; Proctor and Konishi 1997) and projects to the telencephalic nucleus field L, which in turn sends major projections to the AAr and other telencephalic targets (Cohen et al. 1998). AAr projects back to the auditory space maps in optic tectum (OT) and ICX and also to motor nuclei in the brain stem that mediate head saccades and may, therefore, be considered as output station of the auditory forebrain pathway (Cohen et al. 1998; Knudsen et al. 1995). Neurons in all mentioned forebrain stations have been reported to display specific tuning to ITD and ILD (Cohen and Knudsen 1995, 1996, 1998). Moreover, neurons in the AAr have been shown to be sensitive to broad ranges of frequencies (Cohen and Knudsen 1995). A map of ITD has not been found in the forebrain.

Predictions by an optimal coding model have suggested a different representation of ITD for low (<2.5 kHz) and high
(>2.5 kHz) frequencies for the barn owl (Harper and McAlpine 2004). This prediction generated new interest in low-frequency ITD coding in the barn owl. While the midbrain pathway discards low-frequency information almost completely (Wagner et al. 2007), several studies indicate that low-frequency information is present in the forebrain pathway (Cohen and Knudsen 1996; Perez and Pena 2006). The study by Perez and Pena (2006) indicated that low frequencies contribute to higher variations in ITD representation across frequencies. However, in AAr, the contribution of the entire frequency range available to the owl to overall tuning shape to sound localization cues has not yet been investigated. Early studies have been conducted with high-pass-filtered noise (>4 kHz) and yielded similar tuning properties than the ones found in ICX and the OT (Cohen and Knudsen 1995).

A quantitative comparison between responses of single neurons in AAr and ICX is not available. This report closes this gap. Using broad band noise, we show that AAr neurons display significant differences in tuning to ITD and frequency compared with ICX neurons. Specifically, low-frequency inputs caused increases in asymmetries of ITD tuning curves. We suggest that the different spectral filtering of AAr neurons and their sensitivity to ITD represents an adaptation to special aspects of sound localization supported by the auditory forebrain pathway.

METH O DS

Owl handling

Eight barn owls were used in this study. Animals were kept in aviaries at natural light-dark cycle and fed on 1-day-old chicks. On the day prior to an experiment, owls did not receive any food. On experimental days, anesthesia was initiated as described in the following text, and the bird was wrapped into a cloth jacket to keep body temperature constant. The jacket also served to tether the animal to the experimental holder in an upright position similar to the owl’s natural posture. After an experiment, the owl was kept in a small box so that the recovery was easy to survey. Only when fully awake, was a bird returned to its aviary. Between experiments, the animals were given 10–14 days to recover. The owls’ weight ranged between 410 and 470 g and was controlled on a regular basis before experiments.

Surgery

Before every surgical intervention, animals were anesthetized with an initial intramuscular injection of diazepam (Valium, 1 mg/kg) and ketamine (30 mg/kg). A single dose of atropine sulfate (0.065 mg/kg) was injected to prevent excessive saliva production during anesthesia. Ten minutes before the first surgical cut, the animal received buprenorphine (Temgesic, 0.06 mg/kg) as an analgesic. In a primary surgery, a metal head piece that served to fix the head in later experimental holder in an upright position similar to the owl’s natural posture. After the first penetrations, coordinates relative to the zero point of our stereotactic system were used. While no criteria other than recording depth are known to differentiate between the lateral striatum and the auditory arcopallium, we could exclude any erroneous recordings from the midbrain or Field L based on coordinates and/or physiological properties, such as bursting activity of the neurons in the OT or more caudal coordinates for Field L. We used electrolytic lesions in two owls to confirm the recording sites. The data from ICX units were available from earlier studies in our lab (Wagner et al. 2007). ITD is mapped along the anterior-posterior extent of the ICX. Although sampling in ICX included all locations, a bias to frontal recording areas cannot be ruled out.

Stimulation protocol

White noise (0.1–20 kHz) at varying ITDs was used as a search stimulus. ITD, ILD, and frequency tuning, and binaural rate level functions were assessed as initial characterization of an auditory unit. We presented white-noise stimuli varying in ITD (~270 to 270 µs in 30 µs steps; positive ITDs refer to right ear leading), in ILD (~20 to 20 dB in 4 dB steps; positive ILDs refer to right ear louder), or in average binaural level (10 to 82 dB attenuation in 4 dB steps) to obtain tuning curves for the respective parameter, while all other parameters were held constant.
were kept constant at the unit’s best value. Whenever the average binaural level was not the parameter that was varied, it was fixed at ≈30 dB above the response threshold of a unit. Frequency tuning was characterized using pure tones (500–9,500 Hz in 500 Hz steps). We used data from ICX units to compare with the AAr units. The ICX data were collected for different studies (Wagner et al. 2007) and were acquired with similar protocols. For further characterization of the ITD response of AAr units, we presented filtered noise stimuli using either high-pass \( F_{\text{stop}} = 2.5 \text{ kHz}, \ F_{\text{pass}} = 4 \text{ kHz} \) or low-pass \( F_{\text{stop}} = 4 \text{ kHz}, \ F_{\text{pass}} = 2.5 \text{ kHz} \) filters (finite impulse response, least-squares, 100th order) with \( F_{\text{stop}} \) and \( F_{\text{pass}} \) marking the borders of the transition band. Moreover in some units, ITD tuning with tonal stimuli was measured. We chose frequencies so that the periods were integer multiples of the ITD sampling steps. To prevent adaptation effects, stimuli were presented with an interstimulus interval of 1 s and in random order with a repetition of each stimulus of at least five times.

Data analysis

The basic analysis of the electrophysiological data consisted of calculating the average spontaneous firing rate of a unit during the 400 ms preceding stimulus presentation and the average response firing rate of a unit in a response window of 100 ms after stimulus onset plus response latency. Response latency was calculated for each block of stimulus presentations conducted with one variable parameter. To assess response latency, a histogram of the stimulus response rate over time was calculated. Response latency was defined as the point in time after stimulus onset at which the response rate first reached the half-maximal response level. Binning of the histogram was optimized according to a bootstrap method (Friedman and Priebe 1998).

Tuning parameters

Before the analysis of tuning properties in ITD, ILD, or frequency tuning, the spontaneous rate averaged over all stimulus values was subtracted from the tuning curve. The stimulus value that elicited the peak response was used as a measure of best ITD, ILD, or frequency, respectively. Because many ITD tuning curves showed substantial asymmetries, a side peak could reach the same peak height as the main peak but have lower modulation than the main peak. Therefore to find the main peak, we wrote a MatLab routine that searched for all local maxima at ≥80% of the absolute maximum in the smoothed ITD curve (3-point moving average window). The maximum closest to the absolute minimum of the curve, i.e., showing the steepest modulation, was taken as main peak. We chose the equivalent maximum of the unsmoothed curve as peak ITD. The ITD at the steepest slope was calculated as the average ITD between the peak ITD and the minimum ITD on the side of the peak with the deeper modulation.

Tuning width in ITD and ILD tuning curves was defined as the width of the main response peak at different response levels (25, 50, or 75% of the peak response). If the critical response level was not reached on both sides of the peak, the peak width was not calculated. In frequency tuning curves, the width of tuning was not constricted to the main peak. Many units were responsive to more than one frequency range. To capture the whole extent of the frequency range, tuning width was defined as the number of data points in the tuning curve exceeding a critical response level (10, 25, 50, or 75% of peak response) multiplied by the frequency step size used in stimulation (usually 500 Hz).

In ITD tone curves, i.e., the ITD tuning curves obtained with tonal stimuli, we calculated the best interaural phase difference (IPD) instead of a best ITD, as these curves show phase ambiguity due to the periodic structure of the stimulus. ITD was converted into IPD by dividing each ITD value by the period of the stimulus frequency. Using circular statistics as in Yin and Kuwada (1983), the best IPD was defined as

\[
\text{bestIPD} = \arctan \left( \frac{\sum r(IPD) \sin(IPD)}{\sum r(IPD) \cos(IPD)} \right)
\]

with \( r \) being the average response at a certain IPD.

Asymmetry index

Asymmetry indices were calculated as an additional parameter to characterize ITD noise tuning curves. The minimum response was subtracted from the tuning curve. The asymmetry index was calculated as the difference between the area under the curve right of the main peak and the area under the curve left of the main peak normalized by the whole area. The asymmetry was only evaluated in a section of the curve that contained an equal number of data points \( N \) to the left and right of the main peak. With \( r \) being the response function, the asymmetry index \( \text{AsymI} \) was defined as follows

\[
\text{AsymI} = \frac{\sum_{i=1}^{N} r(\text{ITD}_{\text{peak}+i}) - r(\text{ITD}_{\text{peak}+1})}{\sum_{i=1}^{N} r(\text{ITD}_{\text{peak}+i}) + r(\text{ITD}_{\text{peak}+1})}
\]

The index can take values between 0 and ±1 with a step function having an asymmetry index of 1 or −1 and any axially symmetric curve having an asymmetry index of zero. The index was used both over a short range of ITDs with \( i \leq 4 \) \((i < 4 \text{ if the end of the measured ITD range was reached on one side of the peak})\) and the maximal range of ITDs.

Anatomy

Electrolytic lesions (3 current pulses of duration of 10 s each and amplitude of 3 μA each) were set at central recording sites in two owls. Five to 7 days after lesioning, the owls were deeply anesthetized with pentobarbital and perfused with 4% paraformaldehyde in phosphate buffer. Brains were removed, dissected, and sunk over three days in 30% sucrose with 4% paraformaldehyde in phosphate buffer. Finally, brains were cut into 30 μm frontal sections on a microtome. Brain slices were mounted on slides and Nissl stained.

Results

Recordings in the auditory forebrain were obtained from eight owls. The major part of the data (275 units of 290, 95%) was recorded from four owls. The average recording position was located 10.1 mm deep from the surface of the brain. Recording positions of all forebrain neurons clustered over a range of ±1 mm (SD) in the anterior-posterior axis, ±1.1 mm in the mediolateral axis and ±1.1 mm on the vertical axis from that center location. Figure 1 shows a photograph of a Nissl-stained cross-section of the brain at the level of the arcopallium. The lesions mark the recording positions in the medial portion of the arcopallium, which corresponds to the AAr, the auditory subnucleus of arcopallium.

After semiautomatic spike sorting, neural signals were classified as originating from a single- or multiunit based on visual judgment of the spike wave forms. Of a total of 290 units, 253 were considered single units and 37 multiunits. As the percentage of multi units was small (13%), all units were pooled in a section of the curve that contained an equal number of data points \( N \) to the left and right of the main peak. With \( r \) being the response function, the asymmetry index \( \text{AsymI} \) was defined as follows

\[
\text{AsymI} = \frac{\sum_{i=1}^{N} r(\text{ITD}_{\text{peak}+i}) - r(\text{ITD}_{\text{peak}+1})}{\sum_{i=1}^{N} r(\text{ITD}_{\text{peak}+i}) + r(\text{ITD}_{\text{peak}+1})}
\]

The index can take values between 0 and ±1 with a step function having an asymmetry index of 1 or −1 and any axially symmetric curve having an asymmetry index of zero. The index was used both over a short range of ITDs with \( i \leq 4 \) \((i < 4 \text{ if the end of the measured ITD range was reached on one side of the peak})\) and the maximal range of ITDs.
substantially. Units had a median response latency of 16 ms (lower and upper quartile at 11 and 20 ms), which was lower than the median latencies described in Cohen and Knudsen (1995) but in line with the latencies shown in Reches and Gutfreund (2008).

Tuning curves from 76 ICX neurons were available from earlier studies in our laboratory as well as a peak ITD and best-frequency data from 588 ICX neurons (Wagner et al. 2007).

**ITD tuning**

Tuning to ITD noise stimuli was assessed in 290 units. In 236 units (81%) tuning was significant, i.e., responses changed significantly with varying ITD (Kruskal-Wallis test, \( P < 0.05 \)). Figure 2 (middle column) shows two representative examples of ITD noise tuning curves of different AAr units. Tuning curves displayed a variety of shapes. Some units displayed a symmetric main peak and small side peaks, which is reminiscent of ITD-tuning curves of neurons in the ICX and the optic tectum (OT). However, many units featured asymmetric tuning curves such as the ones shown in Fig. 2. These curves were characterized by a main peak that usually occurred close to 0 \( \mu s \) ITD. To one side of the peak, the steep-slope side, response rate reached a minimal level, typically within \( \sim 60 \mu s \). The steep-slope side typically included 0 \( \mu s \) ITD. To the other side of the peak, the shallow-slope side, decrease in response rate with ITD was less steep. In addition, the response minimum on the shallow-slope side was typically higher than the response minimum on the steep-slope side. The consequence of this response behavior was a function resembling a step function added on a sinusoidally modulated curve.

ITD curves from ICX neurons are known to display symmetric peaks (Takahashi and Konishi 1986). To find out whether there was a significant difference between AAr and ICX neurons, we quantified the degree of asymmetry in the population of ITD curves recorded in AAr (\( n = 236 \)) and ICX (\( n = 76 \)). We first calculated the asymmetry index over a range of four sampling points corresponding to 120 \( \mu s \) to the left and right from the peak of the ITD curve (a smaller range to both sides of the peak was considered if the end of the measured ITD range was reached on 1 side of the peak). Figure 3A shows an example of an ITD curve recorded in AAr with an asymmetry index of 0.25. The curve displayed a steep slope on the side of ipsilateral leading ITDs and a shallow slope on the side of contralateral leading ITDs. The example in Fig. 3B depicts an ITD curve recorded in ICX with an asymmetry index of 0.01. The distribution of asymmetry indices was centered around 0 in ICX units but shifted to positive values in AAr units (Kruskal-Wallis test, \( P < 0.01 \)). Thus in AAr units ITDs leading on the side contralateral to the position of the peak in AAr.
The recording electrode tended to elicit elevated responses (Fig. 3C). Also the absolute values of asymmetry indices were significantly different between AAr units and ICX units (Kruskal-Wallis test, \( P < 0.01 \), Fig. 3D). Asymmetry indices in AAr units were larger than in ICX units (percentiles at 25, 50, and 75% in AAr units: 0.07, 0.15, and 0.25 and in ICX units: 0.03, 0.07, 0.15). If the asymmetry index was calculated over the maximal ITD range to the left and right of the peak, asymmetry indices for AAr units were still larger than in ICX units (Kruskal-Wallis test, \( P < 0.02 \)). The difference was less significant because asymmetries in the height of side peaks as shown in example Fig. 3B influenced the index as well.

Apart from the difference in tuning-curve shape, we found significant differences in peak ITD distribution as well as in tuning width. As illustrated in Fig. 4A, the distribution of peak ITDs from 236 AAr units was narrow around a median of 30 \( \mu \)s (lower and upper quartile at 0 and 60 \( \mu \)s, respectively). Peak ITDs on the ipsilateral side rarely had absolute ITD values exceeding 30 \( \mu \)s, while on the contralateral side values >30 \( \mu \)s were common.

Peak width was measured at 75, 50, and 25% of the peak response rate (Fig. 4, B and C). Peak width increased with decreasing response level. As shown in Fig. 4B, for 226 AAr units, the median peak width at 75% of the peak response was 54 \( \mu \)s (lower and upper quartile at 38 and 75 \( \mu \)s, respectively). In 195 units, the response level fell <50% on both sides of the peak, so that the peak width could be calculated. The median peak width was 95 \( \mu \)s (lower and upper quartile 71 and 132 \( \mu \)s, respectively). Response rates fell <25% of the peak rate in 130 units. The median peak width at this level was widest with 136 \( \mu \)s (lower and upper quartile 101 and 186 \( \mu \)s, respectively). In summary, between 75 and 25% of the peak height, the median peak width augmented in increments of 41 \( \mu \)s at each quarter of peak height, while the variability in the distribution of peak widths increased. Furthermore, with decreasing critical response levels, the number of curves in which the response rates fell below that level on both sides of the peak decreased. This observation was due to the reported asymmetry in ITD noise curves.

For the comparison of peak ITD distributions, data from 588 ICX units were available. The entire ITD tuning curve was available only from a subpopulation of 76 units. Therefore measures of peak width were obtained from 76 ICX units.

Results are represented as white symbols in Fig. 4. The distribution of peak ITD values (15 \( \mu \)s median, 0 and 45 \( \mu \)s lower and upper quartile, respectively) was shifted to smaller values compared with best ITD values from AAr units (Fig. 4A). The difference in the medians between ICX and AAr units was small but significant by the Wilcoxon test (\( P < 0.001 \)).

The peak width was significantly lower in ICX units compared with AAr units at all response levels (Kolmogorov-Smirnov test, \( P < 0.001 \), Fig. 4B). In all units, response rates fell <75% of the peak response level on both sides of the peak. The median peak width at this level was 40 \( \mu \)s (27 and 52 \( \mu \)s lower and upper quartile, respectively). At 50% of the maximum response the median peak width was 67 \( \mu \)s (53 and 84 \( \mu \)s lower and upper quartile, respectively) and could still be measured in 74 units. Peak width augmented to a median of 98 \( \mu \)s (77 and 120 \( \mu \)s lower and upper quartile, respectively) at 25% of the peak response level and was measured in 60 units. The observation that peak widths at low response levels could be measured in a larger proportion of tuning curves was due to a higher symmetry of ITD noise curves around the peak in ICX units. The increments in median peak width with each quarter of the peak level were ~29 \( \mu \)s in ICX units compared with 41 \( \mu \)s increments in AAr units. Figure 4C illustrates the resulting difference in peak width between AAr and ICX units.

**Frequency tuning**

The shape of the ITD noise curve depends on the frequency tuning of a unit. ITD sensitivity arises in narrowband frequency channels and undergoes important changes as frequency channels converge in higher stations of processing. In the midbrain ICX, the convergence of frequency channels represents a major step in ITD processing, because it removes phase ambiguity. We found significant differences in fre-
We recorded iso-intensity frequency tuning curves of 224 AAr units. Responses of AAr units to pure tones were often less vigorous than responses to broadband noise. Still in many neurons, frequency-tuning curves revealed sensitivity to a broad range of frequencies. Figure 2 (left) shows examples of iso-intensity frequency-tuning curves measured in two different units. Responses to frequencies <3 kHz were common, while peak responses mostly occurred to frequencies >3 kHz. Frequency-tuning curves were often of complex shape and not simply single peaked. As illustrated in Fig. 5A, the distribution of peak frequencies included the whole range of tested frequencies from 500 to 9,500 Hz and was bimodal with a small peak around 2,500 Hz and a bigger peak at 6,500 Hz. Its median was at 5,500 Hz (3,000 and 6,750 Hz at lower and upper quartile, respectively). Due to the peculiarities mentioned in tuning-curve shapes, the peak frequency did not always convey useful information about the tuning properties, especially in curves that were rather flat over a certain range of frequencies or were multi-peaked (cf. Fig. 2).

The width of frequency tuning was defined as the frequency sampling step (500 Hz) multiplied with the number of frequencies at which the response exceeded a certain response level given as percentage of the maximal response. Tuning width was often of complex shape and not simply single peaked.
was not restricted to peak width because the description of the curve as unimodal was inadequate for many units. As shown in Fig. 5B, tuning width started off narrow at 75% of the maximal response rate with a median of 1 kHz (lower and upper quartile at 1 and 2 kHz, respectively). The median tuning width at the half-maximal response level was 3 kHz (lower and upper quartile at 2 and 4 kHz, respectively). At 25% of maximal response level, tuning width was even broader with a median at 5 kHz (lower and upper quartile at 4 and 6.5 kHz, respectively). Finally, the response range was broadest at 10% of the maximal response with a median of 7 kHz (lower and upper quartile at 5.5 and 8 kHz, respectively).

Peak-frequency values from 588 ICX units were available as well as frequency-tuning curves from 69 ICX units. The white symbols in Fig. 5 represent the frequency-tuning properties of ICX units. The distribution of peak-frequency values extended less to the extreme edges of the frequency range. Most units had peak frequencies >2.5 kHz. The distribution was significantly different from the distribution of peak frequencies of AAr units (Kolmogorov-Smirnov test, \( P < 0.01 \)) with a median of 6,600 Hz (lower and upper quartile at 5,555 and 7,000 Hz, respectively).

Frequency-tuning width was significantly narrower in ICX units than in AAr units at 10, 25, and 50% of the maximal response rate (Kolmogorov-Smirnov test, \( P < 0.001 \)). At 75% of the maximal rate, the median tuning width of 1 kHz was not significantly different from tuning width in AAr units although the values for ICX units fell into a narrower range (lower and upper quartile at 1 and 1.5 kHz, respectively). At 50% of the maximal rate, the tuning width values for ICX units had a lower median (2 kHz) than AAr units and were distributed over a narrower range (lower and upper quartile at 1.5 and 3 kHz, respectively). Naturally, tuning width increased when measured at lower response levels. At 25% of the maximal response, the median was at 3.5 kHz and lower and upper quartiles were at 2.5 and 4.5 kHz, respectively, whereas at 10% of the maximal response, the median was at 4.5 kHz and the lower and upper quartile were at 3.5 and 5.6 kHz, respectively. However, the increment of tuning width at 25 and 10% of the maximal rate was smaller in ICX units than in AAr units. Therefore the difference in tuning width between AAr and ICX units became larger as the critical response level decreased. Figure 5C shows a schematic with the width at different response levels corresponding to the median-width values of AAr units (gray peak) and ICX units (white peak). The figure illustrates clearly that AAr units had a broader tuning at lower response levels. It should, however, be kept in mind that the peaked shape does not represent the shape of single frequency-tuning curves.

**ILD tuning and impact of ILD on ITD tuning**

ILD is processed in a separate pathway in the auditory brain stem (Takahashi et al. 1984). ITD and ILD information merge in the lateral shell of the ICC (ICCls) and the ICX in a multiplicative way (Fischer et al. 2007; Mazer 1998; Pena and Konishi 2001). Consequently, responses to ITD can only be obtained if an appropriate ILD is chosen as well and vice versa. However, the shape of the tuning curve is not affected by this interaction. We investigated tuning to ILD in AAr units as well as the influence of ILD on ITD tuning curve shapes.

The sensitivity of units to changes in the ILD of a noise stimulus was tested in 234 AAr units. Response functions to ILD displayed various shapes from almost flat as shown in Fig. 2B (right) to a peaked shape (not shown). Typically, units responded well to ILDs around 0 dB and to either the positive or negative range of ILDs. ILD tuning curves, therefore often resembled saturating, monotonic curves (cf. Fig. 2B).

Peak ILD values were distributed over the whole range of physiological ILDs (Fig. 6A) with a median of 0 dB (lower and upper quartiles: −8 and 8 dB). The width of the ILD tuning was measured at 75, 50, and 25% of the maximal response rate.

**Fig. 6.** ILD tuning in AAr and ICX units. Symbols correspond to those used in Fig. 4. A: distribution of peak-ILD values in AAr and ICX. The distributions were similar (Kolmogorov-Smirnov test, \( P = 0.72 \)). B: width of ILD tuning measured at different response levels. The distributions of peak widths at different response levels (75, 50, and 25% of maximum response rate) were significantly different between AAr and ICX units only for the 75% level (Kolmogorov-Smirnov test, 75% level: \( P = 0.034 \), 25% level: \( P = 0.14 \); asterisk, the distributions that differed significantly). C: median of tuning width at different response levels measured in the AAr population and the ICX population. Note that due to overlap of the distributions, the white area marking the ICX distribution is plotted in the background.
Measures were only taken if the response rate fell under the respective level on both sides of the peak. Due to the open peaked or rather monotonic curves, the number of measures taken decreased with the critical response level. Figure 6B shows the distribution of peak-width values measured at the different levels of response rate, whereas Fig. 6C depicts the medians of the distributions in a schematic of scaled superimposed peaks. Median-peak width decreased with increasing proximity to the peak response. At 25% of the maximal rate, peaks were broad with a median of 23 dB (18 and 26 dB at lower and upper quartile, respectively). The median tuning width decreased to 17 dB (12 and 21 dB at lower and upper quartile, respectively) at the half-maximal response level and further to 8 dB (5 and 12 dB at lower and upper quartile, respectively) at the 75% response level.

ILD curves from 52 ICX units were available for comparison with the AAr data. Results of the analysis of ILD tuning curves from ICX units are shown as white symbols in Fig. 6. The distribution of best ILD values for ICX units was not significantly different from the distribution for AAr units (Kolmogorov-Smirnov test, $P = 0.72$). Also tuning-width values were largely similar at different response levels. Only at 75% of the maximal rate ILD tuning was broader in ICX units than in AAr units (Kolmogorov-Smirnov test, $P = 0.02$).

As mentioned in the preceding text, in ICX, ITD, and ILD tuning are independent and act in a multiplicative manner. In the forebrain pathway, ITD and ILD channels seem to merge partly already in the nucleus ovoidalis (Proctor and Konishi 1997). A dependency would arise if convergence of ILD channels with different ITD sensitivity occurred throughout the processing steps in the forebrain. Such a dependency should entail differences in ITD curves measured at different ILDs involving not only the peak but the whole curve. Therefore we quantified similarities in ITD tuning measured at different ILDs as correlation coefficient $r$ with the ITD curve measured at the units’ best ILD.

ITD tuning curves at different ILDs were recorded in 48 AAr units. Figure 7, A and B, shows an example of a unit’s ILD tuning and ITD tuning curves at different ILDs, respectively. Only ITD curves measured at ILDs that elicited responses equal or higher than the half-maximal response in ILD tuning were considered for analysis. In this unit, ITD curves at different ILDs differed in minimal and maximal response rates but were modulated in a similar way. They all displayed the main peak around 30 ms and a pronounced slope crossing 0 ms. Because the similarity between the curves was expressed as the correlation coefficient $r$ between each of the ITD curves and the ITD curve measured at the unit’s best ILD, $r$ equaled 1 at the unit’s best ILD (Fig. 7D). Correlation typically decreased with increasing distance from the unit’s best ILD. In the example unit, $r$ reached a minimum of about 0.6 at ±8 dB from the unit’s best ILD (0 dB). If ITD curves were smoothed with a 3-point average window as illustrated in Fig. 7C, $r$ was higher at all values of ITD (black line in Fig. 7D). Figure 7E summarizes the results from all 48 units. Correlation coefficients were pooled and averaged for all units according to the ILD difference to the unit’s best ILD. When the difference between the best ILD and the tested ILD was 0 dB, the correlation coefficient was 1, reflecting autocorrelation. Between ±10 dB from the best ILD the mean correlation coefficients varied between 0.6 and 0.85 for the original data and between 0.7 and 0.9 for the smoothed data. This result suggested that ILD had only a weak influence on the modulation of ITD curves.

Impact of high- and low-frequency bands on ITD tuning

ITD sensitivity in the midbrain pathway is a result of across-frequency integration (Mazer 1998; Takahashi and Konishi 1986; Wagner et al. 1987). In a similar way, ITD sensitivity in AAr units may result from ITD sensitivity contributed by different frequency channels. AAr units tended to show increased sensitivity to the frequency range <3 kHz as...
shown in Fig. 5. The observed asymmetries in ITD noise curves (cf. Fig. 3) could originate from ITD tuning in the low-frequency range. To test this hypothesis, ITD-tuning curves were recorded with noise that was high-pass filtered (see METHODS) and changes in asymmetry were quantified. The asymmetry was calculated over the maximal range of ITDs because a potential contribution of the low-frequency spectrum to ITD sensitivity should affect larger parts of the ITD curve than only the points close to the peak. Significant ITD tuning to high-pass-filtered noise was obtained from 53 AAr units. Additionally, ITD tuning to low-pass-filtered noise was assessed in 21 units. On a single-unit basis, changes in the shape of the ITD curves recorded with filtered noise versus broadband noise were subtle and rarely significant by the Kolmogorov-Smirnov test (6 units of 53 for high-pass-filtered noise and 2 of 21 for low-pass-filtered noise, P < 0.05). Figure 8, A–C, shows examples of ITD curves recorded with broadband noise and filtered noise. As is obvious from the insets, all units were responsive to frequencies <3 kHz. Although the ITD tuning of the unit in Fig. 8A did not change appreciably at stimulation with high-pass-filtered noise, the unit in Fig. 8B responded to a narrower range of ITDs when the noise was high-pass filtered. As a consequence, the ITD curve was less asymmetric. The unit in Fig. 8C had an almost step-like ITD tuning when stimulated with broadband noise. The tuning curve remained asymmetric but lost its step-like shape when high-pass-filtered noise was used. With low-pass-filtered noise, the peak of the curve was shifted to more contralateral leading ITDs. The differences in the distributions of asymmetry indices (Fig. 8D) resulting from stimulation with filtered noises compared with stimulation with broadband noise were not significant by the paired signed-rank test (paired Wilcoxon test, P = 0.052 for broadband vs. high-pass-filtered noise (n = 53), P > 0.3 for broadband versus low-pass and high-pass vs. low-pass-filtered noise (n = 21)). Yet the distributions of asymmetry indices obtained for broadband versus high-pass-filtered noise differed significantly (Kruskal-Wallis test, P < 0.01). Thus the use of high-pass-filtered noise led to more symmetric ITD curves on the population basis. Notably the distribution of asymmetry indices obtained with high-pass-filtered noise in AAr units was not significantly different from the one obtained with unfiltered broadband noise in ICX units (Kruskal-Wallis test, P = 0.81). Hence, low frequencies seemed to have an impact on the asymmetry of ITD curves in AAr units.

**Steep-slope sides of the ITD curve**

As the asymmetry index is maximal for a step function, it is a combined measure of two features that we observed in the ITD curves in AAr: the steep slope and the general bias for mostly the contralateral side of ITDs. We examined the impact of different frequency bands on both features in more detail. In ITD curves from AAr units, the steep-slope side, defined as the flank of the main peak with the highest rate modulation, was generally located around small values of ITD. Figure 9A shows the distribution of the slope ITD, defined as the center ITD between the peak and the trough bordering the steepslope. The distribution of slope ITDs in AAr was narrow with a median of 0 μs (lower and upper quartiles: 30 and 15 μs) and significantly different from the slope-ITD distribution in ICX neurons (Kolmogorov-Smirnov test, P < 0.001). Slope-ITD distribution in ICX neurons was much wider and extended further to the contralateral side (median: 37.5 μs, lower and upper quartiles: −15 and 75 μs), supporting the observation that the steepest slope in these generally more symmetric curves occurred unsystematically on either side of the peak.

**FIG. 8.** Asymmetry of ITD curves recorded with filtered noise. A–C: examples of ITD curves recorded with broadband noise (black line), high-pass-filtered noise (F<hbox>stop</hbox> = 2.5 kHz, F<hbox>pass</hbox> = 4 kHz; gray line), and low-pass-filtered noise (F<hbox>stop</hbox> = 4 kHz, F<hbox>pass</hbox> = 2.5 kHz; light gray line) for 3 AAr units. Insets: frequency tuning curve of each unit. Spontaneous rates were not plotted for reasons of clarity. D: asymmetry indices of ITD curves collected with broadband noise (n = 236), high-pass-filtered noise (n = 53) and low-pass-filtered noise (n = 21) in AAr units. Although asymmetry indices obtained with broadband noise and high-pass-filtered noise differed significantly (Kruskal-Wallis test, P < 0.01), the direction of change in asymmetry using high-pass-filtered noise was not significant (paired Wilcoxon test, P = 0.052). AsymI, asymmetry index calculated over the maximal ITD range.
We wondered whether the high- and low-frequency components in the stimulus affected the steepness of the slope differently. We calculated the steepness of the slope as the ratio of the response-rate difference between peak and trough over the ITD difference. The steepness of the slope was not systematically affected by using high-pass-filtered noise (Wilcoxon paired test, \( P < 0.001 \), Fig. 9B). But slopes were shallower in ITD curves recorded with low-pass-filtered noise compared with the broadband case (Wilcoxon paired test, \( P < 0.001 \)). As slopes in the broadband case were as steep as in the high-pass case but steeper than for low-pass stimuli, the high-frequency fraction in the broadband noise seemed to have a prevailing effect on the steepness of the slope of the ITD curve.

**ITD tuning across frequencies**

In both examples of Fig. 8, B and C, the curve recorded with the broadband noise stimulus seemed even steeper than the one recorded with the high-pass stimulus. The low-pass fraction might even contribute positively to make the slope steeper if its trough was placed close to the peak ITD of the ITD response to high-pass stimuli. This would imply that the neuron was tuned to different ITDs at different frequencies. If neurons were tuned to high values of ITD in the low-frequency range and to small values of ITD in the high-frequency range, this could explain not only the steeper slope observed in Fig. 8, B and C, but also the second prominent feature of the ITD curves in AAr, i.e., the general bias to contralateral ITDs.

We tested this hypothesis by recording ITD curves to tones of different frequencies. Similar to the midbrain, these curves were periodic due to the phase ambiguity inherent in the stimulus (Fig. 10A). In 72 of 92 neurons, we obtained significant tuning to an interaural phase difference (IPD) value for at least one stimulation frequency (Rayleigh test, \( P < 0.001 \)). From each tuning curve we extracted the best IPD and multiplied it with the period of the stimulation frequency to obtain the ITD at the peak closest to 0 \( \mu \text{s} \) ITD. Figure 10B illustrates the ITD values obtained at each stimulation frequency. Because only two neurons had best ITDs on the ipsilateral side, we collapsed ipsi- and contralateral ITDs in this figure. At frequencies between 2,300 and 7,000 Hz, all possible ITDs were represented. Because we used the peak closest to 0 \( \mu \text{s} \)
ITD, the highest possible ITD in each frequency channel was equal to half the period of the stimulus (the $\pi$-limit). At frequencies $> 7,000$ Hz, only ITDs smaller than approximately one-third of the ITD range were represented including $0 \mu s$ ITD. For frequencies $< 2,300$ Hz, ITDs seemed to cluster around one-third of the period. No unit was tuned to $0 \mu s$ ITD. Data from the nine neurons that contributed at least one data point $< 2,300$ Hz were replotted in Fig. 10C with each symbol corresponding to data from one unit. For each of these units the best ITDs changed across frequencies and clustered around a point. Data from the nine neurons that contributed at least one data point in the frequency range (Wagner et al. 2002, 2007). Within the inferior colliculus, the number of neurons with best frequencies have been observed on both the anatomical and physiological levels (Carr and Boudreau 1991; Koepp and Carr 1997; Koepp et al. 1993). Sensitivity to ITD is observed within almost the whole frequency range (Wagner et al. 2002, 2007). Within the inferior colliculus, the number of neurons with best frequencies decreased from core of the ICC (ICCo) to ICCLs and dropped even more in ICX (Wagner et al. 2007). The lowest characteristic frequencies observed in OT were 2 kHz (Knudsen 1984). Thus low-frequency information may be said to be lost in the midbrain pathway.

One of the characteristics of ITD tuning in the first binaural stations is the phase ambiguity inherent in the ITD curves. Phase ambiguity results from the narrow frequency tuning of these neurons. In such neurons the veridical ITD cannot be discriminated from phantom ITDs lying multiples of periods from the veridical ITD (Saberi et al. 1997; Takahashi and Konishi 1986). This problem is resolved in the midbrain pathway in the projection from ICCLs to ICX by a specific way of across-frequency integration: frequency channels converging on one target neuron share one best ITD. As a result ICX units exhibit a symmetric main peak and lower side peaks (Mazer 1998; Saberi et al. 1999; Pena and Konishi 2000; Takahashi and Konishi 1986; Wagner et al. 1987).

Across-frequency integration occurs also in the forebrain pathway. Moreover, this pathway, diverging from the midbrain pathway at the level of ICC, conserves low-frequency information as shown for Ov and Field L (Cohen and Knudsen 1996; Perez and Pena 2006; Proctor and Konishi 1997) and for AAr in the present study. Phase ambiguous ITD curves in response to noise stimuli constituted $42\%$ of ITD tuning curves in Ov (Perez and Pena 2006) but were rarely observed in our AAr population. Across-frequency integration resulted in asymmetric ITD curves with a steep slope toward the main peak close to $0 \mu s$ ITD and a shallow slope for ITDs representing contralateral locations in AAr units. Asymmetric ITD curves may be obtained if different frequency bands contribute sensitivity to different values of ITD. Indeed the overall symmetry of the ITD curves was significantly higher when high-pass-filtered noise was used. This observation fits with conclusions of Perez and Pena (2006), who reported that low frequencies caused inconsistency of ITD tuning across frequency channels in Ov neurons. In our sample of AAr neurons, frequencies $< 2,300$ Hz contributed sensitivity to large values of contralateral ITDs, whereas the high-frequency fraction accounted for the steep slope close to $0 \mu s$ ITD.

AAr neurons typically did not respond well to low frequencies. Because we did not control for a possible dependence of ILD on frequency (Arthur 2004; Keller et al. 1998), we may have used inappropriate ILDs with low-frequency stimuli. However, this should only have diminished the response and not changed the overall shape of the ITD tuning curve as indicated by our data on the influence of ILD on ITD tuning (Fig. 7).

**ITD representation and behavior**

ITD is the cue that specifies the azimuthal location of a sound source (Keller et al. 1998; von Campenhausen and Wagner 2006), and ITD is the sole cue used by the barn owl to determine the amplitude of azimuthal head saccades toward auditory targets (Poggiati and Conlon 2001). Both the midbrain and the forebrain pathways mediate sound localization, but they seem to have different functions (Cohen and Knudsen 1998; Knudsen et al. 1993; Reches and Gutfriend 2008; Wagner 1993; Winkowski and Knudsen 2007). The midbrain pathway mediates precise sound localization (Knudsen et al. 1993; Saberi et al. 1999; Wagner 1993). Because low frequencies are not represented in the midbrain space map (Wagner et al. 2007), they seem not to be necessary for precise sound localization. One reason could be that at low frequencies the owl’s interaural canal becomes less effective at attenuating sounds (Moisell and Konishi 1981) and possibly alters the range of realized ITDs. Von Campenhausen and Wagner (2006) demonstrated that the available ITD range at frequencies between 1 and 4 kHz is reduced by 14% from $\pm 290 \mu s$ at higher frequencies to $\pm 250 \mu s$ available between 1 and 4 kHz. We think that a reduction by 14% of the available ITD range should not be detrimental for the use of ITDs in the low-frequency range. Although we did not control for the effect of
direct transmission of low-frequency stimuli via the interaural canal, we infer from the data of von Campenhausen and Wagner (2006) that the canal effect caused only small alterations in the realized ITDs in our experiment.

After inactivation of the optic tectum, the latency of head saccades was longer and localization errors were larger (Knudsen and Knudsen 1996; Knudsen et al. 1993). Furthermore, in an attentional cueing paradigm, response latency was longer when the cue pointed to the wrong side than when the cue pointed to the later target side (Johnen et al. 2001). A similar latency shift resulted when, with virtual stimulation, the ITD pointed to a location in one hemisphere, while all other spatial cues pointed to the other hemisphere (Poganiatz et al. 2001). These observations led to the proposal that the forebrain pathway may provide an attentional bias signal for the activity of the downstream targets. Some of the characteristics of such a signal have been described. For example, data by Winkowski and Knudsen (2007) demonstrated a matched topographic connection between AAr and OT that could function as top-down control for attention guidance. We speculate that the low frequencies represented in the forebrain might provide a spatial bias signal of the laterality of the stimulus. An advantage would be that phase ambiguities do not occur for frequencies <2 kHz. In response to a brief sound occurring on one side of the animal, a large population of AAr neurons can be activated and might cue midbrain activity in one hemisphere to allow for enhanced processing of consecutive stimuli on that side thereby providing a shift in spatial attention. It needs, however, to be tested whether this is really the case. In line with this speculation, recent data demonstrate that owls are able to localize low-frequency stimuli in a free-flight experiment, although performance was better if the stimulus contained high frequencies (DTT Plachta, personal communication).

Recently a role of AAr in novelty detection has been proposed (Reches and Gutfreund 2008). One cue involved was ITD. This is consistent with the possible role attributed to ITD as a cue more generally informative in the context of auditory scene analysis and not so much as a localization cue (Joris and Yin 2007; Joris et al. 2006).

Implications on ITD coding

Apart from the low-frequency representation, a small but significant difference in the distribution of ITD peaks was observed between AAr and ICX. The ITD peaks in AAr were shifted to larger contralateral ITDs. We think it very unlikely, but cannot totally exclude, that this difference was due to a sampling bias to frontal areas in the ICX. We like to point out that these differences, albeit interesting, do not have a strong implication with respect to coding when considered separately from other observations. In AAr, however, and not in ICX, the ITD at steepest slope was scattered narrowly around zero. These two observations, the slopes at zero combined with the peaks at contralateral ITDs suggest a common ITD curve shape characteristic of a majority of AAr neurons. More importantly, this common feature can be explained by a frequency-dependent distribution of ITD peaks in AAr. The interaural phase differences were scattered around a constant value, 0.3, for frequencies <2.300 Hz. The best phase of 0.3 caused the steepest slope to cross zero ITD as demonstrated by the median shown in Fig. 9A. This had not been observed in any subnucleus of the inferior colliculus of the barn owl (Wagner et al. 2007). For example, in our data set from ICX, the median was at 37.5 μs (Fig. 9A). Whereas in ICX tuning to frequencies <2,500 Hz was rare, in ICC, core phase tuning in ITD tone curves showed a continuous distribution including all phases between 0 and π at frequencies between 500 and 9,000 Hz (Wagner et al. 2007; their Fig. 9).

The concentration of mean interaural phase differences at 0.3 is reminiscent of observations in small mammals (Brand et al. 2002; Coffey et al. 2006; McAlpine et al. 2001; Pecka et al. 2008; Yin et al. 1986) as well as model predictions based on optimal coding (Harper and McAlpine 2004). These data were interpreted as indicative of a slope-coding mechanism. The observations from AAr we present here therefore implicate that in AAr a slope-coding mechanisms may be at work as well.

We mention in passing that, in contrast to the situation in small mammals, where the peak ITDs typically lay outside the physiological range and only the slopes lay inside (Skotun 1998; McAlpine et al. 2001), the barn owl’s broader head and the relatively high-frequency range causes the peak responses to occur within the physiological ITD range and the steep slope to cover the central part of the physiological range.

What roles might the peaks and slopes in ITD curves of AAr neurons play in ITD coding? The peaks might signal the absolute values of ITD, as they do, for example, in orientation tuning (Blasdel 1992). The corresponding slopes, being steepest close to 0 μs ITD, may be useful in discrimination tasks (Seung and Sompolinsky 1993). In AAr units, both features, peaks and steepest slopes, fell within the physiological range. Therefore both might be important. Butts and Goldmann (2006) showed that both peak and slope codes may be realized at the same time depending on experimental context and on response variability. We speculate that the prominent slopes observed in AAr might be useful in signaling small differences in ITD at center ITDs. Another possibility would be that the steep slopes point to sensitivity to moving stimuli at center ITDs. This could be tested in future experiments. Moreover the asymmetry, resulting in general enhanced responses to contralateral ITDs, could serve as a reliable population signal of stimulus laterality. We like to point out that a topographic connection (Winkowski and Knudsen 2007) and our speculation of a population code for laterality and fine discrimination need not be mutually exclusive. Different parts of the available information may be read out in parallel.

We are aware that slope information in midbrain neurons has been correlated to discrimination performance by Bala et al. (2003). Their behavioral data were collected in intact animals, thus reflecting the joined contributions of midbrain and forebrain pathway to discrimination performance. Our data on the slopes of ITD functions suggest that the auditory forebrain pathway could provide an important signal for discriminating center ITDs. Therefore our observations of differences in tuning between the AAr and ICX that go far beyond earlier reported differences in mapping (Cohen and Knudsen 1999) shed new light on the different functions of these two pathways.
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