Estimated Cochlear Delays in Low Best-Frequency Neurons in the Barn Owl Cannot Explain Coding of Interaural Time Difference

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1Institute for Biology II, Department of Zoology and Animal Physiology, Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany; 2Group for Neural Theory, Department d’Etudes Cognitives, École Normale Supérieure, Paris, France; and 3Laboratoire de Neurosciences Cognitives, INSERM U960, Paris, France

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Singheiser M, Fischer BJ, Wagner H. Estimated cochlear delays in low best-frequency neurons in the barn owl cannot explain coding of interaural time difference. J Neurophysiol 104: 1946–1954, 2010. First published August 11, 2010; doi:10.1152/jn.00501.2010. The functional role of the low-frequency range (<3 kHz) in barn owl hearing is not well understood. Here, it was tested whether cochlear delays could explain the representation of interaural time difference (ITD) in this frequency range. Recordings were obtained from neurons in the core of the central nucleus of the inferior colliculus. The response of these neurons varied with the ITD of the stimulus. The response peak shared by all neurons in a dorsoventral penetration was called the array-specific ITD and served as criterion for the representation of a given ITD in a neuron. Array-specific ITDs were widely distributed. Isolevel frequency response functions obtained with binaural, contralateral, and ipsilateral stimulation exhibited a clear response peak and the accompanying frequency was called the best frequency. The data were tested with respect to predictions of a model, the stereausis model, assuming cochlear delays as source for the best ITD of a neuron. According to this model, different cochlear delays determined by mismatches between the ipsilateral and contralateral best frequencies are the source for the ITD in a binaural neuron. The mismatch should depend on the best frequency and the best ITD. The predictions of the stereausis model were not fulfilled in the low best-frequency neurons analyzed here. It is concluded that cochlear delays are not responsible for the representation of best ITD in the barn owl.

IN T RO D U C T I O N

At present, the representation of interaural time difference (ITD) is controversial (Brand et al. 2002; Carr et al. 2009; Harper and McAlpine 2004; Joris et al. 2006; Leibold 2010; McAlpine and Grotze 2003; McAlpine et al. 2001; Palmer et al. 2007; Pecka et al. 2008, 2010; Tollin and Yin 2005; van der Heijden and Joris 2010; Vonderschen and Wagner 2009; Wagner et al. 2002, 2007). Three possible mechanisms have been suggested for this representation (for a review see Joris and Yin 2007). Briefly, Jeffress (1948) proposed the existence of a network in which many ITDs are represented in an array of neurons. These neurons use the coincident arrival of spikes from the two brain sides to compute ITD in a central-auditory nucleus. External delays are compensated for internally by delay lines arising at the input to the coincidence-detector neurons. A systematic variation in the interaural delays leads to the formation of a map of ITD. We refer to this model as the “place-code model”. A second model, the “stereausis model,” was proposed by Shamma et al. (1989). These authors suggested that binaural differences in the excitation of cochlear locations could provide the interaural delays required for coincidence detection. This model does not require central delay lines, but uses a central, binaural nucleus for coincidence detection (for more details see following text). Finally, it was pointed out that only two broad, hemispheric channels that act in a push–pull manner may underlie the representation of auditory space (McAlpine et al. 2001). These two channels would also work in a frequency-specific way and would mathematically reflect coincidence detection at a particular interaural phase difference. We refer to this latter model as the “slope-code model”. Harper and McAlpine (2004) applied optimal-detection theory to the problem of ITD representation. They predicted an implementation of the slope-code model in the low-frequency range and a map of ITD in the high-frequency range.

The available data favor the realization of the slope-code model in many mammals (Brand et al. 2002; Harper and McAlpine 2004; Lane and Delgutte 2005; McAlpine et al. 2001; Pecka et al. 2008, 2010). Cochlear mismatches as required in the stereausis model could be shown in the cat for low frequencies (Joris et al. 2006). No evidence for the stereausis model was found in high-frequency neurons (>3 kHz) of the barn owl (Pena et al. 2001). Moreover, data from the barn owl were in line with the place-code model, but did not support the predictions of the slope-code model (Carr and Konishi 1988, 1990; Sullivan and Konishi 1984; Wagner et al. 2007). It remained unclear, however, why in low-frequency neurons (<3 kHz) of the barn owl array-specific ITDs may occur outside the physiological ITD range of this bird (>±250–280 μs; Haussmann et al. 2009; Keller et al. 1998; Poganiatz et al. 2001; von Campenhausen and Wagner 2006; Vonderschen and Wagner 2009; Wagner et al. 2007). This led to the question of whether these large delays in the low-frequency range may be caused by stereausis.

Prerequisite for coincidence detection in the stereausis model is a central array of coincidence-detector neurons that receives direct or indirect binaural input from the tonotopically organized ipsi- and contralateral basilar membranes (see Fig. 1). Sound entering the cochlea elicits a traveling wave along the basilar membrane that first reaches and excites locations representing high frequencies at the base and subsequently locations representing low frequencies at the apex (Gummer et al. 1987; von Békésy 1960). A matrix of binaural coincidence detectors receives phase-locked inputs from both ears via the auditory nerve and the cochlear nucleus. These inputs are...
snapshots of the excitation pattern of both basilar membranes and depend on the ITD of the stimulus (Fig. 1). At the coincidence detector, the instantaneous spatial cross-correlation between the simultaneous input patterns from the two ears is computed. If there is no time disparity between the input to the two ears at the basilar membrane, the traveling waves of both basilar membranes are identical (Fig. 1A, bottom) and coincident input occurs at the diagonal of the stereausis network according to the characteristic frequencies (CFs) of each basilar membrane. A time disparity between both ears (Fig. 1B) leads to a frequency disparity at the two basilar membranes, since the ipsilateral ear becomes excited slightly earlier than the contralateral ear. Thus the traveling wave in the contralateral ear is delayed with respect to the ipsilateral ear and the excitation pattern of the basilar membranes is phase shifted (Fig. 1B, bottom). This creates a spatial mismatch between the two input patterns in the stereausis network that can be used to estimate the spatial position of a sound source.

In birds, hair cells are contacted by eighth-nerve fibers that send their afferent information to the cochlear nucleus. The cochlear nucleus magnocellularis (NM) projects bilaterally to nucleus laminaris (NL), the first binaural nucleus in the ascending auditory pathway. NL is the station in which coincidence detection takes place in distinct frequency bands (Carr and Konishi 1988, 1990; Seidl et al. 2010; Slej et al. 2010; Sullivan and Konishi 1984; Young and Rubel 1986). NL projects to the core of the central nucleus of the inferior colliculus (ICCc) (Takahashi and Konishi 1988). ICCc neurons exhibit a reduction in response variability compared with NL neurons (Christianson and Pena 2006, 2007), but otherwise resemble in their responses NL neurons, specifically with respect to both frequency and ITD tuning (Carr and Konishi 1990; Pena et al. 1996; Vieite et al. 1997; Wagner et al. 2002). Because of the similarity of response properties between NL and ICCc, it was possible to analyze low-frequency ITD tuning in the owl using activity recorded in ICCc.

We investigated whether the stereausis model could account for the observed frequency and ITD sensitivity of neurons with low best frequencies (BFs) in the barn owl’s ICCc. The data presented below argue against stereausis in the barn owl, an accepted model for sound localization.

**METHODS**

**Owl handling and surgery**

The data were obtained from four barn owls (Tyto alba pratincola) of both sexes taken from the breeding colony of the Institute for Biology II at RWTH Aachen University. All procedures were in accordance with the National Institutes of Health guidelines for animal experimentation and approved by the Landespräsidium für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (Recklinghausen, Germany). On the day prior to an experiment, the weight of the owl was monitored and the bird did not receive any food but had free access to water. On experimental days the owl’s weight was controlled again. Sedation was introduced by an intramuscular (im) injection of ketamine (Valium, 1 mg/kg; Ratiopharm, Ulm, Germany). Anesthesia was initiated after a waiting period of about 30 min by applying an im injection of ketamine (30 mg/kg; Sanof-Ceva, Düsseldorf, Germany). A single dose of atropine sulfate (0.065 mg/kg; B Braun AG, Tuttingen, Germany) was administered to prevent saliva secretion during anesthesia. Buprenorphine (Tengesics, 0.06 mg/kg; Essex Pharma, Munich, Germany) was given im as an analgesic.

As described earlier, a metal head plate was fixed onto the skull of the owl under anesthesia before it was used in electrophysiological experiments (e.g., Vonderschen and Wagner 2009). During experiments, the posterior edge of the head plate served as a stereotactic zero coordinate for both the rostrocaudal and the mediolateral axis. To allow penetrations of the electrodes, a craniotomy was made over the recording site, which was sealed with petroleum jelly (Vaseline) during experiments and which was covered with antibacterial ointment (Nebacetin; Astellas Pharma, Munich, Germany) and dental cement (Paladur). After the experiment had been terminated. During experiments, anesthesia was maintained with intramuscular injections of diazepam and ketamine as required. After terminating the experiment, a second injection of buprenorphine was administered before the skull was sutured and the wound was treated with antibacterial ointment (Nebacetin). The owl was removed from the setup and brought to a recovery box, where it was provided with food and was easy to monitor. Only when fully awake and showing no evidence of discomfort the owls were returned to their aviary. Each owl was used more than once.

**Signal generation and data acquisition**

Experiments were conducted in a soundproof anechoic chamber (IAC 403A; Industrial Acoustic, Niederkrüchten, Germany). Acoustic signals consisted of 100 ms long dichotic noise or pure tone signals...
with 5 ms on- and offset cosine ramps. Signals were generated using Visual C++ software (Microsoft, Redmond, WA) in combination with Brainwave (Tucker-Davis Technologies; TDT, Alachua, FL) on a personal computer (PC). Digital to analog (DA) conversion was maintained using a DA converter (DA 34, System II; TDT) at a sampling rate of 50 kHz. DA-converted signals were individually attenuated for the left and right channels by programmable attenuators (PA4; TDT). Antialias filtering of the signals was done using an FT6 (TDT). Finally, the signals were power-amplified (Yamaha AX 590) and presented through calibrated earphones (Sony MDR-E831LP). Epoxide-ylt-insulated tungsten microelectrodes (18 – 25 MΩ; FHC, Bowdoinham, ME) were used for recording. The recorded electrophysiological signals were preamplified (M. Walsh Electronics, Pasadena, CA), impedance matched, amplified and filtered (50-Hz notch filter; 300 – 5,000 Hz band-pass filtered; M. Walsh Electronics), analog to digital converted (25 kHz, AD1; TDT), and read into a PC. Preanalysis of the data and on-line spike sorting was done with Brainwave (TDT). Final data analysis was performed off-line with self-written Matlab routines (The MathWorks, Natick, MA) and GraphPad Prism (La Jolla, CA).

Stimulation protocol

Broadband noises (0.1 – 20 kHz) or tonal signals were used for stimulation. Cells were initially characterized by their responses to ITD (‘ITD tuning curve’); varying step size; positive ITD indicates right ear leading), interaural level difference (ILD; ‘ILD tuning curve’; – 20 to 20 dB, step size 4 dB; positive ILDs refer to right ear louder), frequency (“isolevel frequency response function” or frequency–response curve, 200 to 9,200 Hz, step size 500 Hz), and level (“rate-vs.-level [RLF] function”), binaural and monaural paradigm, average binaural level at best ITD and ILD between 0 and 72 dB attenuation). During the recordings, the stimuli were presented once/s in random order. Each stimulus was repeated five to ten times. These recordings served to classify a response as originating from ICCc (for details see next section).

After this initial physiological characterization of the unit, recordings of higher resolution were performed for further analyses: ITD step size was adjusted in accordance with the unit’s BF and varied from 30 to 250 μs, so that four to six ITDs per period were presented. Furthermore, frequency–response curves were recorded with a step size of either 50 or 100 Hz to achieve a high resolution of the resulting frequency-tuning curve. The average level for binaural as well as monaural frequency–response curves was kept at about 30 dB above threshold of the unit’s BF similar to Pena et al. (2001).

Physiological characterization of the location of recording

All recordings were made from the ICCc. Neurons were recorded from both sides of the brain. Coarse targeting of the nucleus was achieved by known stereotactic coordinates with reference to the head plate (~8 – 9 mm caudal, ~2 – 4 mm lateral, 12 – 15 mm below brain surface). The final classification was achieved by comparing the response characteristics with established physiological criteria for the ICCc and for neighboring nuclei (lateral shell of the central nucleus of the inferior colliculus: ICCls, medial shell of the central nucleus of the inferior colliculus: ICCMs, external nucleus of the inferior colliculus: ICX; see supplemental material in Wagner et al. 2007). In brief, both ICCc and ICCls show a cyclic ITD-tuning curve to stimulation with broadband noise, with no suppression of the side peaks compared with the main peak, whereas ICX neurons exhibit side-peak suppression. Neurons in ICCMs are not tuned to ITD. ILD-tuning curves in ICCc are flat, whereas ICCc neurons display a sigmoid ILD tuning favoring contralateral ILDs. Frequency–response curves in ICCls and ICCc show a tonotopy from low to high frequencies with increasing recording depth and a tuning width, typically in the range of 1/3 of an octave at frequencies > 1.5 kHz (see following text). Stimuli from both ears excite neurons in ICCc (EE property), whereas neurons in ICCls show excitation to contralateral stimulation and inhibition to ipsilateral stimulation (EI property). Along the frequency axis, neurons in ICCc and ICCls exhibit functional ITD arrays. The response peak shared by all neurons in a dorsoventral penetration, the array-specific ITD, served as a criterion for the representation of a given ITD in a neuron. The sign of the array-specific ITD corresponds to the sign of the stimulus ITD for neurons in ICCc (“ipsi arrays”), whereas the signs are different for neurons in ICCls (“contra arrays”). The array-specific ITD was used in the final classification because it had turned out earlier to be the most stable classification criterion (Wagner et al. 1987, 2002, 2007).

Data analysis

We determined several characteristics of a cell’s response. 1) The average spontaneous discharge rate recorded during the 400-ms period preceding the stimulus. 2) The average response rate during stimulation minus the spontaneous rate. The response was calculated in an interval of 100 ms after stimulus onset and compensation for the response latency. 3) Frequency–response curves were characterized by three parameters. The first parameter was the BF of the unit, which is the frequency eliciting maximum response in the frequency–response curve. We also calculated the width at half height of the tuning curve (W50). W50 corresponds to the range of frequencies over which the discharge rate of the unit corresponds to ±50% of the maximum discharge rate (Pena et al. 2001; Wagner et al. 2002). Since BF may depend on sampling resolution, we also determined the center frequency at 50% of the maximum discharge rate (BF50).

Computational model

We used a cross-correlation model to determine the predicted relationship between ITD and bilateral frequency mismatch under the stereausis model (Shamma et al. 1999). The cross-correlation model was used in two ways. First, we used the model to compute the predicted ITD given the measured monaural best frequencies. Second, we used the measured ITD and ipsilateral best frequency to compute the predicted contralateral best frequency and thus the predicted bilateral frequency mismatch. For each prediction, ITD-sensitive neurons were modeled using a modified version of the Bonham and Lewis (1999) cross-correlation model, as in Pena et al. (2001).

To compute the predicted ITD for a given bilateral frequency mismatch, an input signal was filtered with two gammatone impulse responses and the resulting signals were cross-correlated. The input stimulus was a broadband noise signal with a flat spectrum between 0 and 8 kHz at a sampling rate of 100 kHz. The ipsilateral and contralateral gammatone filters had impulse responses given by

\[ g_{\text{ip}}(t) = \left[ t - t_0(f_i) \right] \exp\left\{ -\left[ t - t_0(f_i) \right] / \tau(f_i) \right\} \times \sin\left\{ 2\pi f_i [ t - t_0(f_i) ] \right\} H(t - t_0(f_i)) \]  

\[ g_{\text{con}}(t) = \left[ t - t_0(f_i) \right] \exp\left\{ -\left[ t - t_0(f_i) \right] / \tau(f_i) \right\} \times \sin\left\{ 2\pi f_i [ t - t_0(f_i) ] \right\} H(t - t_0(f_i)) \]  

respectively, where \( H(t) \) is the unit step function. The cochlear delay is a frequency-dependent parameter that was taken from latencies of auditory nerve fibers in the barn owl and is given by \( t_0(f) = 3.04 - 0.22 \ln(1000f) \) (Köppl 1997). The frequency-dependent time constant \( \tau \) was taken from Carney and Yin (1988) as \( \tau(f) = 1.3 / (0.456 + 0.8) - 2.585 + 0.4(0.456 + 0.8) - 0.3447 \). The time constant \( \tau \) is given in milliseconds and the frequency \( f \) is given in kilohertz. Since the time constant \( \tau \) was derived from the cat, we changed \( \tau \) by an order of magnitude in the simulations to test for robustness of the data. We found no significant differences in the results. The cross-correlation was performed in Matlab (MathWorks) using the function xcorr.
Because the input signals were not purely sinusoidal, the cross-correlation had a single dominant peak and the best ITD was given by the ITD at the peak of the cross-correlation.

To derive the predicted bilateral frequency mismatch for a given ITD, we first computed the mapping between ITD and bilateral frequency mismatch for a fixed value of the ipsilateral best frequency. We fixed the value of the ipsilateral best frequency at the measured value and, for a range of values of the contralateral best frequency, computed the ITD at the peak of the cross-correlation as described earlier. This produced a mapping between ITD and contralateral best frequency or, equivalently, between ITD and bilateral frequency mismatch. The predicted value of the contralateral best frequency under the stereausis model was found by interpolating this mapping at the measured ITD. The predicted bilateral frequency mismatch was then given by the difference between the measured ipsilateral best frequency and the predicted contralateral best frequency.

**RESULTS**

For this project, recordings from 44 sites in the low-frequency region (<3 kHz) of the ICCc of four barn owls were collected. Of these 44 sites, 11 were well-isolated single units, whereas the rest were classified as multiunits, all showing homogeneous spike forms. The responses of single and multiunits were similar and showed no significant differences in the tuning parameters analyzed. Therefore the data sets of the units were pooled for all analyses. In the following section, we will first describe the general tuning properties of the recorded units and then compare the obtained results with predictions of the stereausis model.

**General response properties**

To determine whether binaural cochlear delays play a role in the representation of ITD, both ITD and frequency tuning are important. Therefore we characterized the responses of the individual cells when ITD or frequency was varied. Generally, when the ITD of a broadband stimulus was altered, the neurons exhibited cyclic ITD tuning (Fig. 2, A and E). The period of the cyclic response was close to the period derived from the best frequency (BF) of the neuron (1/BF; see dashed lines in Fig. 2, A and E). We did the analyses with the best ITD derived from the ITD shared by all neurons in a dorsalventral array, termed the array-specific ITD (Wagner et al. 1987, 2007). We also used the ITDs that were closest to zero in the ITD-tuning curves for comparative analyses. We generally found similar results with the latter ITD sample as with the array-specific ITDs.

Frequency–response curves typically exhibited a clearly identifiable peak with steep slopes on both sides of the BF (Fig. 2, B, D, and F–H). The frequency at the peak response was termed the BF of a neuron. Stimulation with frequencies far away from BF sometimes evoked inhibition, as indicated by the negative spike rates in Fig. 2. Binaural frequency–response curves (Fig. 2, B and F) were generally more similar to contralateral (Fig. 2, D and H) than to ipsilateral response curves (Fig. 2, C and G). When the discharge rates of the whole sample were considered and single and multiunits were compared, we found no significant differences in discharge rate between these two populations (t-test for the binaural paradigm and Mann–Whitney tests for the monaural paradigms, all P > 0.05). However, differences in discharge rates evoked by binaural or monaural stimulations were obvious in some cases (Fig. 2, B–D and F–H) and discharge rates evoked by binaural and ipsilateral stimulation, as well as by contralateral and ipsilateral stimulation, differed significantly (Wilcoxon-matched pairs test, P < 0.004), with ipsilateral responses being lower. No significant difference was found between binaural and contralateral response rates (Wilcoxon-matched pairs test, P = 0.104).
We did not observe significant differences in frequency-tuning parameters between single and multiunits (t-test, all \( P > 0.05 \)). Response latencies between single and multiunits, as well as between binaural and monaural paradigms, were not significantly different (Mann–Whitney and t-test, Kruskal–Wallis, and Dunn’s post test).

Distribution of best ITDs

In the stereausis model, the required frequency mismatch depends on the best ITD. Therefore we attempted to record neurons with different best ITDs. In our sample, 23 units had their array-specific ITD peak within the barn owl’s physiological ITD range (±280 \( \mu \text{s} \); Hausmann et al. 2009; Keller et al. 1998; von Campenhausen and Wagner 2006). In the remaining 21 units the array-specific ITD was outside the physiological ITD range. Extreme best ITDs were as low as \( -1,000 \mu \text{s} \) or as high as \( +800 \mu \text{s} \) (Fig. 3A). The units with array-specific ITDs outside the physiological ITD range were particularly interesting because clear frequency mismatches were expected if stereausis would be responsible for the shift of the best ITD away from 0 \( \mu \text{s} \).

Measures and distribution of best frequencies

The two measures of best frequency, BF and BF\(_{50}\), were not significantly different for all stimulus paradigms [Fig. 3B; binaural: \( P = 0.626 \) (Wilcoxon matched-pairs test); ipsilateral: \( P = 0.426 \); contralateral: \( P = 0.226 \) (paired t-test for monaural paradigms)]. Additionally, when BF\(_{50}\) was plotted as a function of BF, the slopes of the linear regression for all comparisons were close to one (Fig. 3B, inset) and the correlations between BF\(_{50}\) and BF were high (binaural: \( r^2 = 0.977 \); ipsilateral: \( r^2 = 0.988 \); contralateral: \( r^2 = 0.973 \)). Since BF and BF\(_{50}\) were almost identical, the results indicated that frequency-tuning curves typically showed a highly symmetric shape (Fig. 2, B, D, and F–H). For all further analyses we exclusively used the BF\(_{50}\) values.

The BF\(_{50}\) values followed a normal distribution for the monaural stimulus paradigms (Kolmogorov–Smirnov [KS] test, all \( P > 0.05 \)), but not for the binaural paradigm (Fig. 3C; KS test, \( P = 0.033 \)). A Kruskal–Wallis test yielded no significant differences in the median of BF\(_{50}\) values for all paradigms (\( P = 0.921 \)). Best binaural frequencies in this sample ranged from 0.408 to 2.793 kHz.

Frequency mismatch between contralateral and ipsilateral inputs

The stereausis hypothesis predicts a frequency mismatch between the contralateral and ipsilateral inputs that depends on both the BF and the best ITD of a particular neuron (Shamma et al. 1989). For an ITD of 0 \( \mu \text{s} \), the BF\(_{50}\) values in the ipsi- and contralateral frequency–response curve should be almost identical. For best ITDs different from zero the expected frequency mismatch between the ipsi- and contralateral side should increase with the absolute values of the best ITD. Therefore we plotted the measured frequency mismatch (\( \text{BF}_{50,\text{ipsi}} - \text{BF}_{50,\text{contra}} \)) as a function of measured array-specific ITD (Fig. 4) to test for a systematic change of frequency mismatches. The correlation between frequency mismatch and array-specific ITD was low (\( r^2 = 0.02 \)) and no systematic change of frequency mismatch with ITD could be observed.

In further analyses, we investigated the effect of frequency mismatches more closely. We generally observed no significant differences in BF\(_{50}\) between ipsi- and contralateral stimulation (paired t-test, \( P = 0.0558 \)). For the next analysis we divided the sample in two populations (Fig. 5A). One population included all units with an array-specific ITD within the barn owl’s physiological ITD range of ±280 \( \mu \text{s} \). The other

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Distributions of array-specific ITDs and best frequencies. A: the number of neurons binned according to their array-specific ITD (bin size of 100 \( \mu \text{s} \)). Twenty-three neurons had their array-specific ITD within the physiological range of the barn owl (±280 \( \mu \text{s} \)), whereas 21 units had their best ITD outside this range. B: the BF\(_{50}\) (kHz) value of each neuron as a function of BF (kHz). The resulting regression for each stimulus paradigm is indicated in the inset. The regression lines are plotted in addition. Values are written as inset. C: distribution of BF\(_{50}\) values for the 3 stimulus paradigms (bin size: 0.2 kHz; black: binaural; dark gray: ipsilateral; light gray: contralateral). The numbers on the x-axis in A and C denote the centers of the bins.
population was composed of all neurons having their array-specific ITD outside this range. The latter neurons typically had BF$_{50}$ < 2 kHz and, in fact, most best frequencies (14 of 21) were <1.0 kHz. In contrast, the BF$_{50}$ values of neurons with an array-specific ITD within the physiological ITD range were scattered over a range of BF$_{50}$ > 1 kHz. The regression analysis between the ipsi- and contralateral BF$_{50}$ yielded the following results (in kHz): BF$_{50,\text{contralateral}}$ = 0.84 × BF$_{50,\text{ipsilateral}}$ + 0.4448 for neurons with an array-specific ITD within the physiological ITD range ($r^2 = 0.68$). Since the slope (0.84) was <1, the BF$_{50}$ values of the inputs from the two sides became more similar as BF$_{50}$ decreased. A similar relation held for the neurons having their array-specific ITD outside the physiological ITD range: BF$_{50,\text{contralateral}}$ = 0.9063 × BF$_{50,\text{ipsilateral}}$ + 0.1138 (kHz, $r^2 = 0.97$). Below 1 kHz, there were almost no differences between the ipsi- and contralateral inputs.

Next, we compared the frequency mismatches of the same neurons, but separated those into populations of neurons with either negative or positive array-specific ITDs (Fig. 5B). The neurons with negative best ITDs had a mean frequency mismatch (BF$_{50,\text{ipsi}}$ − BF$_{50,\text{contra}}$) of −0.0012 kHz and a median mismatch of −0.0362 kHz. Similar results were found for the neurons with positive best ITDs. Here, mean and median frequency mismatches were 0.1047 kHz and −0.0754 kHz, respectively. A Mann–Whitney test revealed no significant differences ($P = 0.770$) between frequency mismatches of units with negative and positive array-specific ITDs. This finding was supported by the slopes as well as the $y$-intercepts of the linear regressions for both samples, which did not differ significantly (ANCOVA, $P > 0.05$).

Implications of the stereausis model

We used a cross-correlation model to quantitatively investigate whether the coding of best ITDs as demonstrated so far could be achieved by a computation as described in the stereausis model (Pena et al. 2001; Shamma et al. 1989; see METHODS for further details). In a first approach, the BF$_{50}$ and the measured mismatches in BF$_{50}$ between the ipsi- and contralateral frequency–response curves of a particular neuron were used as input to the model. From these parameters, we predicted the best ITD under the stereausis model. The predicted and measured best ITDs showed a weak correlation ($r^2 = 0.06$; Fig. 6A). Moreover, the predicted and the measured ITDs often showed different signs (34% of all recording sites). In general, the stereausis model predicted best ITDs that were much closer to 0 $\mu$s than were the actually measured best ITDs.

In a second step, the same model was applied using the measured array-specific ITD and the best frequency of a neuron as input. The frequency mismatch in BF$_{50}$ required for stereausis to code the neuron’s particular best ITD was calculated. Since the cochlear representation of best delays depends on the logarithm of the best frequency, neurons having higher BF$_{50}$ values require a larger mismatch in frequency compared with neurons with lower BF$_{50}$ values, if the ITD is held constant. For small ITDs, the stereausis model predicts a small mismatch in frequency. In contrast, the measured mismatches varied over a large range (Fig. 6B). For example, in unit O72U019 (Fig. 6B, black filled circle), having a small array-specific ITD (30 $\mu$s) and medium BF (binaural BF$_{50}$: 1.91 kHz), the measured mismatch was 1.02 kHz, whereas the predicted mismatch was 0.06 kHz. On the other hand, in neuron O72U054 (Fig. 6B, gray filled circle), which had a similar BF$_{50}$ (binaural BF$_{50}$: 2.05 kHz) and an array-specific ITD of −300 $\mu$s, the measured frequency mismatch was 0.06 kHz, whereas the model predicted a mismatch of −0.31 kHz. Again, measured and predicted frequency mismatches were

![Figure 4: Frequency mismatch in ICCc (core of the central nucleus of the inferior colliculus) neurons. The frequency mismatch (kHz) between the left and right ears is plotted as a function of array-specific ITD (μs) and is not correlated with the measured ITD ($r^2 = 0.02$).

![Figure 5: Relationship between ipsilateral and contralateral inputs. A: open gray diamonds represent units with their array-specific ITD within the physiological range of the barn owl. Open black circles show neurons with their best ITD outside this range. Solid lines show the linear regression for both populations, respectively. The slopes were not significantly different and the correlation in both populations was high ($r^2 > 0.68$). The regression equations are given in the inset. B: gray downward oriented triangles represent units with negative array-specific ITDs, whereas black open squares show neurons with positive array-specific ITD. Linear regressions are depicted color-coded as well. The slopes were not significantly different and the correlation in both populations was high ($r^2 > 0.80$). The regression equations are given in the inset.]
often of different signs (31.82% of all recording sites). For example, unit O72U80 had a positive array-specific ITD of 800 µs and a negative measured frequency mismatch, whereas unit O21U163 had a negative ITD of −1,000 µs but a positive measured frequency mismatch. In total, only 1.4% of the variability between measured and predicted frequency mismatches could be explained by a linear regression.

Stereausis predicts large frequency mismatches for units with best ITDs outside the physiological ITD range. However, the measured frequency mismatches were typically in the same range as that for the units having best ITDs within the physiological ITD range (Fig. 6B) and the mismatches often had a sign opposite to the prediction of stereausis.

Comparison of frequency mismatches

Finally, we compared the measured frequency mismatches between the ipsi- and contralateral inputs to the frequency mismatches predicted by the stereausis model as a function of recorded array-specific ITD. As may be seen in Fig. 7, we found substantial differences between measured (black open circles) and predicted (gray filled circles) mismatches for our sample of 44 recording sites. Measured frequency mismatches clustered around ±0.3 kHz, with the exception of two recordings sites at array-specific ITDs of +30 and +250 µs. In contrast, predicted frequency mismatches varied systematically with ITD. The slope of the linear regression for the measured frequency mismatch (solid black line) revealed no significant difference from zero (ANCOVA, P = 0.36), whereas for the predicted frequency mismatches the slope (solid gray line) differed significantly from zero (ANCOVA, P < 0.0001). A comparison of the slopes for both measured and predicted frequency mismatches resulted in a significant difference as well (ANCOVA, P = 0.0014). A similar analysis with the recorded and predicted frequency mismatches of high-frequency neurons recorded in NL (Pena et al. 2001) yielded similar results (data not shown).

DISCUSSION

We investigated ITD- and frequency-response functions in low best-frequency neurons (<3 kHz) of the barn owl’s ICCc in relation to possible mechanisms of sound localization as described by the stereausis model. We observed no systematic frequency mismatch between the ipsi- and contralateral inputs to ITD-sensitive neurons with different array-specific ITDs. In the following we shall first discuss the basic physiological data before we relate our findings to the stereausis model.

ITD and frequency tuning

ITD-tuning curves in single and multiunits were not different (Fig. 2). Such observations had been made before in ICCc (Wagner 1990; Wagner et al. 1987). Both samples showed cyclic ITD tuning, where the period depended on the BF (Wagner et al. 2002). Array-specific ITDs covering a wide range were observed (Fig. 3A). Several low best-frequency units had array-specific ITDs at 0 µs. This is consistent with earlier reports (Wagner et al. 2002, 2007) and confirms the conclusion drawn in these earlier studies that the data do not follow the predictions of the slope-code model (Harper and McAlpine 2004). Similarly, a considerable percentage of neurons had array-specific ITDs outside the physiological ITD range of the barn owl (Fig. 3A), as also reported earlier.
Although we do not yet know why such neurons occur, we can now exclude differences in cochlear delays as the source of these large best ITDs.

The frequency tuning observed (Fig. 2) also resembled earlier observations (Wagner et al. 2002, 2007). Specifically, very low best-frequency neurons (down to a BF of 200 Hz) can be observed in ICCc (Fig. 3, B and C). This is lower than BFs measured in other collicular subnuclei like the neighboring ICCl (Wagner et al. 2007) and similar to the lower limit seen in the eighth nerve, NM or NL (Carr and Köppl 2004; Köppl 1997). Therefore the ICCc is ideal for studying low-frequency effects. BF and BF50 measures did not exhibit significant differences (Fig. 3B). Therefore we used BF50 for the analysis, as was also done by Pena et al. (2001). The typical tuning was primary-like as in mammals (cat: Aitkin et al. 1975; Kuwada et al. 1984; chinchilla: Langner et al. 2002; Nuding et al. 1999; gerbil: Semple and Kitzes 1985; guinea pig: Popelár and Syka 1982; mouse: Yan et al. 2005; rat: Kelly et al. 1991). Also, ipsilateral inputs tended to be weaker and narrower in bandwidth than contralateral inputs (data not shown), as observed in gerbils (Semple and Kitzes 1985).

Comparison with the stereausis model

The stereausis model predicts a frequency mismatch between the ipsi- and contralateral input to a binaural neuron if the best ITD differs from 0 μs (see Fig. 1). This mismatch needs to increase systematically with increasing best ITD of a neuron (Shamma et al. 1989). Our analysis yielded no differences between ipsi- and contralateral BF50 values (Figs. 4 and 5, A and B). Similar observations were made in several other preparations. For example, Aitkin and Reynolds (1975) and Kuwada et al. (1984) also found no differences in frequency tuning between ipsi- and contralateral inputs to neurons in the inferior colliculus of cats. The same holds for the study by Köppl and Carr (2008) who investigated low-best-frequency neurons (1–2.5 kHz) in the chicken NL. On the other hand, Semple and Kitzes (1985) found significantly lower BFs in response to ipsilateral than contralateral stimulation in gerbil IC. The difference was small and it remains unclear whether the difference may be explained by cochlear delays because the corresponding ITD tuning curves are not available. Pena et al. (2001) observed significantly lower ipsilateral than contralateral BFs in high-frequency neurons in the barn owl’s NL. However, the frequency mismatch was not large enough to produce the observed ITD tuning and did not always exhibit the sign expected from the stereausis model. Therefore Pena et al. (2001) concluded that stereausis cannot explain the representation of ITDs in high-frequency neurons of barn owls. Similar observations were also made for low-best-frequency neurons in the alligator. Carr et al. (2009), where the calculated frequency mismatch was not correlated with best ITD. This is also true for our analyses, since frequency mismatch was not correlated with ITD for low-frequency neurons in the barn owl’s ICCc (Figs. 4 and 7).

The available data thus suggest that stereausis cannot explain the array-specific ITDs observed in ICCc of the barn owl. To substantiate this conclusion, we applied a model of stereausis to our data. The quantitative predictions of the model were far off the measured data. Neither did the predicted ITDs match the measured best ITDs (Fig. 6A), nor did the predicted frequency mismatches comply with the measured differences (Fig. 6B). A comparison of measured and predicted frequency mismatches (Fig. 7) supported this conclusion. Furthermore, the distribution of frequency mismatches had a median value of 0.083 kHz at 1 kHz (data not shown) that converts to about 2% of cochlear length or to <1/10 of an octave (Köppl et al. 1993). Such a low-frequency mismatch seems to require mechanisms other than genetics to be established.

In summary, both in the high-frequency range (Fischer and Pena 2009; Pena et al. 2001) and in the low-frequency range (this study), stereausis is not responsible for the coding of ITD in the barn owl.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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