Binaural processing by the gecko auditory periphery

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Binaural processing by the gecko auditory periphery. J Neurophysiol 105: 1992–2004, 2011. First published February 16, 2010; doi:10.1152/jn.00004.2011.—Lizards have highly directional ears, owing to strong acoustical coupling of the eardrums and almost perfect sound transmission from the contralateral ear. To investigate the neural processing of this remarkable tympanic directional sensitivity, we combined biophysical measurements of eardrum motion in the Tokay gecko with neurophysiological recordings from the auditory nerve. Laser vibrometry shows that their ear is a two-input system with approximately unity interaural transmission gain at the peak frequency (~1.6 kHz). Median interaural delays are 260 μs, almost three times larger than predicted from gecko head size, suggesting interaural transmission may be boosted by resonances in the large, open mouth cavity (Vossen et al. 2010). Auditory nerve recordings are sensitive to both interaural time differences (ITD) and interaural level differences (ILD), reflecting the acoustical interactions of direct and indirect sound components at the eardrum. Best ITD and click delays match interaural transmission delays, with a range of 200–500 μs. Inserting a mold in the mouth cavity blocks ITD and ILD sensitivity. Thus the neural response accurately reflects tympanic directionality, and most neurons in the auditory pathway should be directional.

processing of directional information is a major function of the auditory system. In birds and mammals, directional cues such as interaural time differences (ITD) and interaural level differences (ILD) are computed by binaural comparisons in the central nervous system (CNS), and binaural pathways are a major feature of their auditory pathways. The salience of binaural cues has led to specializations at every level of the auditory processing chain, as evidenced in both birds and mammals (Grothe et al. 2005, 2010; Köppl 2009).

In other tetrapods, like frogs and toads, lizards, crocodiles, and some birds, sound can travel relatively unobstructed from one ear to the other (Christensen-Dalsgaard 2010). Such acoustical coupling allows the direct component of sound at the external surface of the eardrum to interact with the indirect component at the internal surface to reduce or enhance tympanic motion (Fig. 1C). Tympanic coupling can therefore enhance the directionality of the ear (Wever 1978; Feng 1980; Calford and Piddington 1988; for reviews, see Michelsen 1998; Hoy et al. 2000; Klump 2000; Feng and Christensen-Dalsgaard 2007). Acoustical coupling is greatest in lizards, which can have interaural transmission gains approaching 0 dB, and directionality differences up to 40 dB (Christensen-Dalsgaard and Manley 2005, 2008). The directionality is frequency dependent, but in lizards, the directional bandwidth is relatively large, ranging from 2 to 6 kHz in the species studied (Christensen-Dalsgaard and Manley 2005, 2008). Since lizards have such well-developed pressure-receiver ears, they could be a model for the study of directional processing (Köppl 2009). However, can they or do they use the same algorithms as other animals?

In birds and mammals, there is ongoing discussion of what algorithms are used for computation of ITDs and ILDs (Köppl 2009; Grothe et al. 2010). It appears that birds use computed sensory maps or place codes, where the individual neurons that make up sensory maps respond maximally to different preferred values of ITD or ILD (Konishi 2003; Wagner et al. 2007). In small mammals like the gerbil, however, the range of physiological ITDs often is not well represented by peak firing of low best frequency neurons (Pecka et al. 2008). Instead, McAlpine et al. (2001) proposed that the azimuthal position of a sound source could be computed from the overall discharge rate within the broadly tuned ITD channel on one side of the brain. Thus, for a sound moving away from the midline, activity will increase in the contralateral hemisphere, toward the peak of the ITD functions, indicating that the sound source has shifted to a more lateral position (McAlpine et al. 2001). Excitatory-inhibitory (EI) neurons sensitive to ILD would produce similar lateralized effects in the brain (Tollin 2003).

Small changes in location are best represented by changes in rate (Takahashi et al. 2003), and changes in rate may be used to direct motor behavior, such as orientation (Groh and Sparks 1992; Campbell et al. 2006). In geckos, rate-based processing of direction could simplify the subsequent CNS processing, because lateralized differences in rate could control the motor output on the two sides of the animal and steer it effectively towards the sound source, as shown by robotic simulations (Shaikh et al. 2009). To test this idea, it is necessary to investigate if the auditory nerve responses are directional. We report on the results of a combined anatomical, biophysical, and neurophysiological investigation of the processing of directional information in the Tokay gecko, up to the level of the auditory nerve, and show that responses in all auditory nerve fibers accurately reflect the strong interaural coupling.

METHODS

This study was based on data from adult Gekko geko of both sexes. All animal care and anesthesia procedures followed procedures approved by the University of Maryland Animal Care And Use Committee and by the Danish Animal Experimentation Board (Dyresognsstyrelsen).

Head reconstruction and buccal molds. To determine the volume and shape of the buccal and middle ear cavity, a perfused gecko head was decalcified, embedded in celloidin, sectioned at 200 μm, and then reconstructed with the aid of the StereoInvestigator module from

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NeuroLucida (MicroBrightfield, Williston VT). Head tissues (skin, buccal cavity, esophagus, brain case, and trachea) were outlined to create a three-dimensional reconstruction. Acoustical transmission between the two ears was blocked by filling the buccal cavity with ear mold compound (Gold Velvet II; All American Mold Laboratories, Oklahoma City, OK). The mouth cavity casts were later weighed to determine their volume. Before use, each mold was carved so it did not impede columellar motion.

Mold efficacy was checked by comparing thresholds of auditory nerve fibers to ipsi- and contralateral stimulation and/or by measuring the sound transmission from one coupler to the contralateral microphone with and without the inserted mold (see Figs. 1D and 10). We measured the transfer functions between sound stimulus and microphone recording at both ipsi- and contralateral microphones and calculated the cross talk as difference between the two transfer functions. The difference between the cross talk with and without block is a measure of the efficiency of the block under the assumption that the acoustics of the preparation did not change between measurements. The calculation of cross talk was done by a DSP with AD and DA converters [RM2 Tucker-Davis system 3; Tucker Davis Technology (TDT), Gainesville, FL] and customized software (CrossTalk). Initial attempts to block transmission by opening the mouth were not effective.

**Laser vibrometry.** Five geckos, weight 24–70 g, were lightly anesthetized by ketamine (10 mg/kg Ketalar; Warner-Lambert/Parke-Davis) and placed in the center of an anechoic room. Ketamine was used instead of the isoflurane used in the neurophysiological experiments, to avoid ventilating the animals, since the intubation might disturb the free sound field. Stimulation and data recording were controlled by TDT system 2 hardware and customized software (DragonQuest). Stimuli were frequency sweeps (175 ms, 200–7,500 Hz, 16 sweeps, levels of 80–90 dB SPL) emitted in an anechoic room from 12 JBL 1-G loudspeakers placed at 30° intervals around the lizard, each at 1 meter distance. It should be noted that geckos have an acoustic reflex comparable to the stapedius reflex (Wever 1978), and we have shown in an earlier study that geckos had nonlinear responses at high sound levels that we attributed to this reflex (Christensen-Dalsgaard and Manley 2005). In the present experiments, however, we did not see departures from linearity. The room has been tested to be anechoic to <200 Hz. However, some reflections, especially from the laser setup, are probably unavoidable and may explain the spectral ripple in some of the measurements. The signal sent to the loudspeakers was deconvoluted with the individual loudspeakers characteristics (measured with a B&K 0.5-in. microphone at the center of the setup before placing the animal) by dividing the spectrum of the sweep by the transfer function of the speaker. For local ipsi- and contralateral stimulation, we used a Beyer DT-48A headphone in a coupler placed ~0.5 cm from the eardrum but not sealing the eardrum. With both free-field and local stimulation, the sound at the animal’s eardrum was measured with a B&K 4182 probe microphone, digitized (22-kHz sample rate, 8,192 samples) using the TDT AD-converter (AD2), and stored in a PC (Christensen-Dalsgaard and Manley 2008). For local stimulation, the nonstimulated ear was partially shielded using baseline barriers, and the direct sound transmission around the head was measured with the probe microphone at the nonstimulated ear and found to be reduced by ≥20 dB. Eardrum vibrations were measured by either a Dantec (Skovlunde, Denmark) laser Doppler vibrometer or (in the later experiments) a Polytec (Walldbronn, Germany) vibrometer OV-505, and we obtained strong reflections directly (no added reflector) from the tip of the extracolumellar attachment close to the centre of the eardrum. Sound and laser recordings were averaged over 16 presentations. From the local stimulation experiments, the interaural transmission gain could be calculated by dividing contralateral eardrum vibration transfer functions by ipsilateral eardrum vibration transfer functions (see Christensen-Dalsgaard and Manley 2008).

**Neurophysiology.** In vivo recordings from 22 geckos were used to investigate the physiology and morphology of the auditory nerve. Head widths from members of this group, measured from earflap to the open middle ear, and the buccal cavity, were 25.6 ± 5.3 mm (n = 10). Anesthesia was induced by 3% isoflurane inhalation via a mask, followed by intubation. Body temperature was maintained at 26°C by a heating blanket wrapped around the animal. A constant gas flow of carbogen mixed with 1–3% isoflurane at 2–4 ml/min was connected via a long loose fitting tube around the animal. A constant gas flow of carbogen mixed with 1–3% isoflurane at 2–4 ml/min was connected via a long loose fitting tube into the trachea; respiration continued under these circumstances. The head was held in a constant position by gluing a stainless steel head post to the prefrontal bone. A dorsal craniotomy exposed the cerebellum and the central portion of the eighth nerve. Most data were obtained with tungsten microelectrodes (F. Haer, Bowdoin, ME), with...
impedances ∼20 MΩ. Electrodes were positioned above the nerve and advanced remotely. We continuously tested for auditory responses using a variety of monaural and binaural stimuli. Electrodes were coupled to a preamplifier and amplifier system (µA200; Walsh Electronics); the amplified signal was high-pass filtered at 300 Hz and fed to an A/D converter (TDT DD1) with subsequent event counter (TDT ET1). Both the analog and the TTL signal were stored and processed by custom-written software (xdphys, Caltech, Pasadena CA).

Recordings were made in a sound-attenuating chamber (IAC, Hannover, MD). Closed, custom-made sound systems were placed at the entrance of both ear canals, containing commercial miniature earphones and miniature microphones (Knowles EM 3068). After the sound systems were sealed into the ear canal using Gold Velvet II ear impression material, the sound systems were calibrated individually before the recordings. Acoustic stimuli (tone bursts, clicks, and noises) were digitally generated by the same custom-written software as above, driving a signal-processing system (TDT). Stimuli were generated separately for the two ears by using a TDT AP2 signal processing board. Both channels were then fed to the earphones via D/A converters (TDT DD1), anti-aliasing filters (TDT FT6−2), and attenuators (TDT PA4). Tone bursts had a 100-ms duration (including 5-ms linear ramps) and were presented at a rate of 5/s. We measured monaural isolevel frequency responses and rate-intensity functions at best frequency for both ipsi- and contralateral stimulation, as well as response areas with covarying frequency and level. Condensation clicks had a rectangular form and duration of two samples (equivalent to 41.6 μs). The standard click had 0 dB attenuation relative to 85 dB

![Fig. 2. Laser measures of delay and directionality](http://jn.physiology.org/)

Note that the free-field eardrum directivity data here are transfer functions between eardrum vibration and sound at the eardrum and thus do not show the effects of diffraction. A: cylinder surface plot showing the amplitude of the eardrum vibration velocity transfer function (x-axis: direction; y-axis: frequency, color scale amplitude; dB re 1 mm·s⁻¹·Pa⁻¹). B: cylinder surface plot showing the interaural vibration amplitude difference, color scale amplitude in dB. Vibration amplitude difference plots were generated by subtracting the free-field eardrum vibration transfer function (in dB re 1 mm·s⁻¹·Pa⁻¹) by its reflection along the frontal-caudal axis. C: spectra of eardrum vibration velocity transfer functions for the frontal directions. Scale = dB re 1 mm·s⁻¹·Pa⁻¹. D: polar plot of eardrum transfer function delays, calculated from the phase spectra. Distance between circles is 200 μs.
SPL. The mean of the best frequencies determined from ipsi- and contralateral frequency responses was taken as the frequency for measuring period histograms at 20 dB above threshold and for testing ITD selectivity with dichotic stimuli. ITDs were tested within ±1 stimulus period in steps no larger than 1/10 of the period and stimulus durations of 100 ms. Stimulus levels were between 50 and 80 dB SPL, and generally 10 stimulus repetitions were presented at each ITD. In addition, we measured ITD-ILD response areas by dichotic stimulation with covarying ITD and ILD, usually with three presentations at each ITD-ILD combination.

Analysis of laser data. The quality of the measurements was gauged by calculating the coherence function (the fraction of output power attributed to the stimulus signal), and only measurement values where the coherence function was >0.9 are reported here. The eardrum vibration transfer functions are displayed as cylinder surface plots as described by Christensen-Dalsgaard and Manley (2005, 2008). Cylinder surface plots are interpolated contour plots of amplitude with direction (X, 12 directions) and frequency (1, 500 frequency bands) as independent variables. All phases were referred to the phase at 1,000 Hz, 90° sound direction. We constructed polar plots from the transfer function phase spectra. Vibration amplitude direction plots were generated by subtracting the sound arriving at the contralateral ear from the sound arriving at the ipsilateral ear. Sound at the ipsilateral ear was represented by the free-field eardrum vibration transfer function (in decibels), and sound at the contralateral ear was represented by the reflection of the transfer function along the rostral-caudal axis (see Fig. 2B; Christensen-Dalsgaard and Manley 2005, 2008). The interaural transmission gain was calculated following Christensen-Dalsgaard and Manley (2008) from the ratio of the ipsilateral eardrum transfer function with local sound stimulation at the ipsilateral and contralateral eardrum. Briefly, the ipsilateral eardrum transfer functions to either ipsi- and contralateral stimulation are used to measure the sound arriving at the internal surface of the eardrum: The ratio between the contra- and ipsilateral transfer functions is the gain of the interaural transmission pathway, since the directly transmitted sound component is negligible (at least reduced to at least 20 dB, as stated above). Interaural transmission delays were calculated from the interaural gain phase spectrum by dividing the slope by $2\pi$.

Analysis of neural data. Monaural measures of best frequency were derived by measuring changes in spike rate in response to changing 100-ms tones at a constant level. Monaural period histograms were constructed from 100 repetitions of the tone stimulus. The timing of each spike relative to the zero-crossing of the stimulus was recorded with a temporal resolution of 1 μs (TD, ET). Period histograms, the mean phase with respect to the stimulus, and the vector strength were derived from these data (Goldberg and Brown 1969). ITD was measured three ways; ITD responses were measured at best frequency and were fitted with a cosine function to determine best ITD, defined as the peak closest to zero ITD (Peña et al. 2001). Characteristic delay and characteristic phase were measured, using four or more different frequencies, according to the methods of Yin and Kuwada (1983). Spike rate, as a function of ITD, was fitted with a cosine function at the respective stimulus frequency (Vite et al. 1997) to determine best interaural phase difference (IPD), defined as the peak closest to zero IPD. A linear regression of best IPD as a function of frequency was calculated, the slope of which corresponds to the characteristic delay and the y-intercept to the characteristic phase (Yin and Kuwada 1983).

Finally, click responses were used to measure internal conduction delay through the mouth. Peristimulus time histograms were constructed from responses to monaural click stimulation (Kaplan et al. 1983), and latencies were calculated to be the first bin after the onset of the click exceeding the spontaneous level and were followed by a bin also meeting this criterion, as in Köppl (1997). Click delays between the ears were calculated by subtracting the ipsilateral click delay from the contralateral delay.

**RESULTS**

Middle ear and mouth form a continuous open cavity. We used whole head reconstructions and casts of the mouth to measure the volume and shape of the buccal cavity, including the middle ear (Fig. 1). Accurate reconstructions allowed for numerical calculation of the eigen-frequencies and the prediction of a large internal delay (Vossen et al. 2010). One gecko head was decalcified and reconstructed at 100-μm intervals (Fig. 1B). Reconstruction revealed a large buccal cavity (volume 4.2 ml; Fig. 1B), while measurement of mouth casts had a mean volume of 3.5 ± 0.95 ml (n = 23). The middle ear forms a recess that is continuous with the cavity, creating an unrestricted connection across the mouth, i.e., with no clear constrictions corresponding to Eustachian tubes in mammals (Fig. 1, A, inset, and B). The columella is exposed in the middle ear recess (Fig. 1B) and runs rostrally to insert into the oval window. Caudally, it contacts the extracolumella and tympanum, as described by Werner and Wever (1972), Wever (1978), Saunders et al. (2000), and Werner et al. (2008). On the exterior, the tympanum is thin, large, and recessed (Fig. 1A, insets).

Eardrums are directional and acoustically coupled. Laser measurements of eardrum vibration (Fig. 2A) showed a pronounced directivity with generally smaller responses at contralateral sound directions and a steep gradient across the midline. The maximal ipsilateral to contralateral directional differences were recorded between 1,100 and 1,520 Hz and ranged from 19 to 34 dB (median 27 dB; n = 5), and the spectra changed systematically with frontal direction, as shown in Fig. 2C. The directional bandwidth, defined as the frequency range by $2\pi$.

![Fig. 3. Median interaural transmission gain amplitude (A; dB) and phase (B; radians) from laser vibrometry measurements on 5 geckos (see METHODS for details).](http://jn.physiology.org/doi/abs/10.1152/jn.00941.2010)
band containing directional differences $>3$ dB, was $\sim 3$ kHz. To highlight the cues available for binaural comparison, we divided the directional response by its mirror image, i.e., by subtracting the dB values (Fig. 2B). This procedure emphasized ipsilateral/contralateral asymmetry of the eardrum response. Furthermore, assuming linear decibel-spike rate functions, this operation can be understood as a very simplified model of neural subtraction.

The phase shift of eardrum vibrations shifts systematically with direction, with maximal directional differences of $5.5$ radians and the ipsilateral eardrum generally advanced accordingly with direction, with maximal directional differences of $0.95$, were observed from fibers with low best frequencies, between $300$ and $500$ Hz. Similar decreases in phase locking with increasing best frequency were observed in the alligator lizard (Rose and Weiss 1988) and in the green tree frog (Narins and Hillery 1983; Fig. 4A).

In about a third of recordings from low best frequency nerve fibers, responses showed two evenly spaced peaks within the phase histogram (Fig. 4B, inset; mean phase difference of $183 \pm 18^{\circ}; n = 12$; see Manley et al. 1990). Double peaked responses were observed both with and without blocks inserted in the mouth. Vector strength declined between $500$ and $2,000$ Hz and also showed a large variation that cannot be attributed to peak splitting. Generally, auditory nerve units responded in a primary-like fashion (Fig. 4C). The short intervals of the initial burst may be seen in a peristimulus time histogram and in a raster plot from the same unit (Fig. 4D; also see Johnstone and Johnstone 1969; Eatock et al. 1981).

**Gecko auditory nerve responses are ITD sensitive.** ITD tuning curves were recorded from $64$ fibers with best frequencies between $200$ and $3,600$ Hz (Fig. 5). In all cases we measured best frequency and generally at $20$ dB above threshold. Phase locking decreased with increasing best frequency but remained statistically significant up to $1,300–1,500$ Hz [only vector strength values with a significance level of $0.01$ or below (Rayleigh test) were accepted; see Goldberg and Brown 1969]. The highest vector strengths, $\sim 0.95$, were observed from fibers with low best frequencies, between $300$ and $500$ Hz. Similar decreases in phase locking with increasing best frequency were observed in the alligator lizard (Rose and Weiss 1988) and in the green tree frog (Narins and Hillery 1983; Fig. 4A).

**Auditory nerve encodes the phase of the auditory stimulus.** We report on $67$ auditory nerve units with best frequencies between $0.2$ and $4$ kHz (Fig. 4A). Gecko auditory nerve units responded to both ipsi- and contralateral sound (see Figs. 6–8), and units with best frequencies below $\sim 1,300$ Hz phase locked to the auditory stimulus. Phase locking was measured around best frequency and generally at $20$ dB above threshold. Phase locking decreased with increasing best frequency but remained statistically significant up to $1,300–1,500$ Hz [only vector strength values with a significance level of $0.01$ or below (Rayleigh test) were accepted; see Goldberg and Brown 1969]. The highest vector strengths, $\sim 0.95$, were observed from fibers with low best frequencies, between $300$ and $500$ Hz. Similar decreases in phase locking with increasing best frequency were observed in the alligator lizard (Rose and Weiss 1988) and in the green tree frog (Narins and Hillery 1983; Fig. 4A).

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**Fig. 4. Phase locking in the auditory nerve.**

A: vector strength as recorded from lizards and tree frog and reviewed in Köppl (1997). Light grey line shows the average curve to data from “freestanding” fibers in Rose and Weiss (1988), their Fig. 10; dark grey line shows the average curve to “tectorial” data from Rose and Weiss (1988), their Fig. 12; dashed line shows the average curve to data from Narins and Hillery (1983), their Fig 4; and thick grey line shows our data from the gecko auditory nerve as a function of stimulus frequency in vivo and does not include data from the double peaked period histograms. Each filled dot represents the vector strength calculated in response to a continuous tone, $20$ dB above threshold, and ranging from $60$ to $90$ dB. Solid line connects median values calculated in $0.5$-octave bins. Symbols show individual gecko nerve data points. Triangles show data from Sams-Dodd and Capranica (1994). B: D: responses from a best low frequency auditory nerve fiber to ipsilateral stimulation, $600$ Hz, $70$ dB. B: period histogram with vector strength of $0.76$. Inset: double peaked period histogram for $400$-Hz unit. C: primary-like response with a peristimulus time histograms (PSTHs) at $600$ Hz and $70$ dB SPL. D: dot raster data for the same unit.
We found ITD tuning in all auditory nerve fibers examined. With tonal stimuli, the auditory nerve responses varied cyclically as a function of interaural phase difference. The regular peaks associated with phase locking may be seen in Fig. 4. Measures of ITD responses, click delay, phase delay, and characteristic delay all showed a similar response minimum. Generally, an ITD that evoked a response minimum was observed when the sound in the contralateral ear led the sound in the ipsilateral ear by 200–400 μs [see Fig. 6, C and E, for an example of the time delay between ipsi- and contralateral ears, and see Fig. 5, C–F (arrows), for examples of the ITD response minimum]. We will describe the measurements of ITD response minima and conclude with summaries of measurements of interaural delay obtained using both biophysical and neurophysiological techniques. Comparison of the units in Fig. 5, A and E, revealed the common minimum, although other minima and maxima were generated by tonal stimuli around best frequency, 2π apart.

Auditory nerve units were sensitive to both tones around best frequency and to broadband noise (Fig. 5, A and B). Responses varied in a cyclic manner with the ITD of a sound stimulus, and the period of the ITD response function matched that of the stimulus tone (Fig. 5C). Characteristic delay was calculated by fitting each ITD curve with a cosine for all frequencies tested and then a linear regression of best IPD as a function of frequency. Values for the y-intercept or characteristic phase (CP; 0.24 cycles) and CD (∼124 μs) derived from this regression are given. MP, mean phase. E: ITD (70 dB) delay curves for an auditory nerve fiber with a best frequency of 500 Hz reveals a similar response minimum to the unit in A, ∼200 μs (arrow). F: matching dot raster plot from this low best frequency unit, showing the change in response rate with ITD and the ITD that evokes a response minimum (arrow).
Fig. 6. Binaural interactions in nerve for low best frequencies: A: ITD responses recorded in gecko auditory nerve as a function of stimulus frequency (best frequency = 525 Hz). Interaural delay curves plot the response of this unit for a range of frequencies from 450 to 600 Hz. B: each curve in A was normalized and fit with a cosine for all frequencies tested, to yield a CD of \(-537\) μs. Inset: best IPD (in cycles) determined for each of the curves shown in B, as a function of stimulation frequency (Hz). Solid line shows the linear regression. Slope of the line represents the CD, and the y-intercept represents the CP. Values for CP and CD derived from this regression are given. C: PSTHs from monaural click stimulation of ipsilateral (grey) and contralateral (black) ears, with 20-μs bins. Latency difference between ipsi- and contralateral response maxima was 320 μs. D: ITD responses as a function of interaural level difference. Interaural delay curves plot the response of this unit for a range of ILDs from \(\pm 10\) dB. Note that transmission loss across the mouth at these low frequencies only yields complete cancellation at \(+10\) dB, when the sound in the contralateral ear is 10 dB louder than the ipsilateral ear. E: period histograms showing monaural responses to stimulation of contralateral (left, black, mean phase 0.425, vs. 0.8) and ipsilateral (right, grey, mean phase 0.15, vs. 0.72) ears. Corresponding interaural delay curves from this unit are shown in A (raw), B (cosine fit), and D (0-dB line).

450 to 600 Hz. The characteristic delay was large (\(-537\) μs), while click and mean phase delays were 270 and 330 μs, respectively (Fig. 6, C and E). At low frequencies, interaural transmission was attenuated (transmission gain \(-10\) dB; Fig. 3A), as reflected in the period histograms recorded in response to stimulation of the right ear (grey) and after stimulation of the left or contralateral ear (black; Fig. 6E). Increasing the contralateral sound pressure by 10 dB compensated for the interaural attenuation and produced almost complete cancellation at the ITD response minimum (e.g., compare \(+10\)-dB line with 0-dB line at the ITD minimum near 400 μs; Fig. 6D). Decreasing the level in the contralateral ear reduced ITD sensitivity (\(-10\) dB). At higher best frequencies (1,000–3,000 Hz; see example in Fig. 7), interaural sound transmission was almost completely unattenuated (transmission gain close to 0 dB; Fig. 3A). Recordings from a high best frequency unit (Fig. 7) revealed a characteristic delay of \(-227\) μs and click delays of 165 μs.

Click delays predict the minimum ITD. Responses to click stimuli were used to measure the latency to stimulation of either the contralateral or ipsilateral ear (Kaplan et al. 1983; Figs. 6C, 7C, and 8, A and B). Click response latency was measured from a peristimulus time histograms constructed from responses to monaural click stimulation, with 128 repetitions and 10-μs bin width. Köppl (1997) has defined latency as the timing of the first bin after the onset of the click that exceeds the spontaneous level and that is followed by a bin also meeting this criterion. We measured the latency of this peak and then calculated the click response latency difference by subtracting the ipsilateral ear click response latency from that recorded after stimulation of the contralateral ear (Fig. 8B). Interaural click response delays were not different to the ITD response minimum (\(P < 0.45\), two-tailed t-test; Fig. 9, A and B).

The mean ITD predicted from these click response difference comparisons was 194 ± 63 μs, with a median of 170 μs (\(n = 24\)). Absolute click response latencies for both ipsilateral and contralateral stimulation were 2.20 ± 0.57 ms (\(n = 30\); ipsilateral stimulation) and 2.35 ± 0.62 ms (\(n = 24\); contralateral stimulation; Fig. 8A).

Mean phase and characteristic delays predict the ITD response minimum. The phase difference between the low best frequency monaural phase-locked responses to stimulation of either ipsilateral or contralateral ears should correspond to the transmission delay through the mouth (see example in Fig. 6E). When the contralateral sound stimulus is advanced to nullify the transmission delay, ipsi- and contralateral sounds coincide at the tympanum to cancel tympanic motion and produce a minimum binaural response (Fig. 9, A and C, blue squares). The differences in mean phase predict the ITD response minimum for 14 nerve recordings (Fig. 9A, blue squares). The minimum ITD was predicted by subtracting the ipsilateral mean phase from the contraterally evoked mean phase, which yielded a value of 285 \pm 112 μs (\(n = 14\); median 248 μs). Phase differences between the two ears are such that, to bring peaks into coincidence, the stimulus to the ipsilateral ear must be delayed with respect to the contralateral ear. This “predicted
ITD minimum” was compared with the “observed ITD minimum” obtained from the ITD curves. Observed and predicted peaks are not different (P < 0.13, two-tailed t-test) and centered ~250-µs ITD (Fig. 9, A and B).

With binaural stimulation, auditory nerve responses reach a minimum when the transmission delay across the mouth exactly compensates for the ITD presented through earphones (Fig. 9, green triangles). This difference in travel time is the characteristic delay. We derived characteristic delays by fitting ITD curves to a range of frequencies around best frequency to cosine functions in which peak positions could be precisely measured (Figs. 6B and 7B; Viete et al. 1997). Plotting the mean interaural phase against frequency yields a line whose slope is the frequency-independent ITD or characteristic delay (Rose et al. 1966; Yin and Kuwada 1983; Peña et al. 2001). Figures 5–7 illustrate characteristic delay calculations for a range of auditory nerve fibers with different best frequencies. The ITD response minimum predicted from the characteristic delay measures was 243 ± 117 µs (n = 24; median: 194 µs). Observed minimum ITD values and predicted characteristic delay peaks are not different (P < 0.21, two-tailed t-test).

The previous three paragraphs show that our three different measures of delay across the gecko mouth, click, mean phase, and characteristic delay, yielded similar results. These results were sufficiently similar that data from 22 geckos could be pooled to show predicted delays were congruent with measured ITD minima (Fig. 9, A and B). As shown in Fig. 9B, physiological measures of delay also matched biophysical observations from laser vibrometry measurements (X; Fig. 9A).

Interaural transmission gains. Just as the ITD responses shown above can be used in calculations of interaural delay, a comparison of the sensitivity to ipsilateral and contralateral stimulation provides an estimate of the interaural transmission gain (Fig. 9D). Both ipsilateral and contralateral stimulation generated sigmoidal rate-level functions, and we used the difference in sensitivity between the ipsilateral and contralateral rate-level functions to provide a measure of the interaural transmission gain (example in Fig. 10D, showing a transmission gain of ~6 dB at 1,500 Hz). Alternative measures of transmission gain can also be calculated from comparisons of isolevel frequency responses with ipsi- and contralateral stimulation (Fig. 9D). We converted the spike rates to equivalent decibel levels by reading the level corresponding to the spike rate off the fiber’s rate-level curve at best frequency with ipsilateral stimulation (Feng 1980). Finally, we also estimated the interaural transmission gain from dichotic stimulation while covarying ILD and ITD. This generates one ITD curve for each ILD. The ILD where maximal cancellation is found (i.e., where a certain ITD-ILD combination reduces spike rate to the spontaneous rate) is a measure of the interaural transmission gain (Fig. 6C), under the assumption that ipsi- and contralateral sound inputs to the eardrum are equal here. Figure 9D shows neural transmission gains for all auditory nerve units measured, with the biophysical transmission gain shown for comparison. Neural gains ranged up to 0 dB, with equal sensitivity to stimulation from the ipsilateral and contralateral ear, but were less at low frequencies. (Christensen-Dalsgaard and Manley 2008).

Blocking interaural transmission. The purpose of blocking transmission was directly to demonstrate the effect of interaural coupling on the neural response. The interaural pathways were blocked by inserting a mold in the mouth (Fig. 1D). This blocked dichotic effects such as ITD-ILD modulation (Fig. 10, B–E). The efficiency of the block was determined by measuring the acoustical cross talk through the head (see METHODS for details; Fig. 10A). The cross talk was generally reduced by >20 dB after inserting the block [compare Fig.
10D (no block) with Fig. 10E (block)], and ITD tuning was lost.

**DISCUSSION**

Gecko ears are highly directional (Fig. 2; and Christensen-Dalsgaard and Manley 2005), and these biophysical features are accurately represented in the responses of the auditory nerve. Interaural delays, measured in the nerve by three different methods, are similar to the delays measured by laser vibrometry (Fig. 9, A–C). The three neural measures yield average values (in μs) of 285 for mean phase, 194 for click and 243 for characteristic delay, and 204 for ITD response minima. All measures were comparable to the median transmission delay of 260 μs found in the laser measurements. Also, the neural data show strong coupling between the two eardrums, with almost equal sensitivity to ipsi- and contralateral stimulation over a wide frequency range, and comparable biophysical and neurophysiological transmission gains (Fig. 9D). The observed ITD response minima most likely reflect the cancellation of eardrum motion by direct and indirect sound components impinging on both sides of the eardrum, as shown by the close correspondence between the response minima and the biophysical measures of interaural delay.

In many respects, the gecko auditory nerve responses resemble binaural responses of the binaural auditory pathways in mammals and birds (for reviews, see Klump 2000; Konishi 2003; McAlpine and Grothe 2003; Grothe et al. 2010). Similar ITD-dependent modulation of neural discharges are produced by neural interactions in the avian nucleus laminaris and the mammalian superior olivary nuclei, and the methods for calculation of characteristic delays and mean phases used here have been used previously to characterize ITD sensitivity of such binaural neurons in cat, guinea pig, gerbil, and rabbit among others (Yin and Kuwada 1983; Yin and Chan 1990; Batra et al. 1997; McAlpine et al. 1998; Hancock and Delgutte 2004; Pecka et al. 2008); in owls and chickens (Takahashi and Konishi 1986); and in alligators (Carr et al. 2009).

An important difference between our results in the gecko and the binaural responses recorded in birds and mammals is that gecko nerve responses reflect the interaction of ipsi- and contralateral inputs on the motion of the eardrum and therefore simultaneously encode ITD and ILD. The distinctions we have unmasked between ITD and ILD were imposed by the use of earphones to deliver sound stimuli. With more naturalistic free-field stimulation, auditory nerve responses should simply reflect the strong directionality of the eardrum (Christensen-Dalsgaard and Manley 2005, and present data).

We hypothesize that most or all neurons in the central auditory pathway should be directional, with the possible exception of very low frequencies (Vossen et al. 2010). At high frequencies, where acoustical coupling decreases, ILDs should be generated by sound diffraction (see below). Our predictions are supported by results from free-field stimulation in the torus semicircularis of *Gekko gecko* (Manley 1981). Gecko torus units exhibited directivity with activity almost completely suppressed at ipsilateral angles. Manley (1981) pointed out that these responses could have been generated by both neural inhibition and acoustical interactions. We have shown that the directionality has its origins in the responses of the auditory nerve and would expect the free-field neural response to mirror the directionality of the eardrum, as shown by our preliminary experiments with free-field stimulation of gecko auditory nerve fibers (Christensen-Dalsgaard and Carr 2011) and in free-field recordings in frogs (Feng 1980; Jørgensen and Christensen-Dalsgaard 1997; Ho and Narins 2006).

It is important to investigate auditory responses with naturalistic, free-field stimulation. Generally, in any animal with coupled ears, dichotic stimulation used to characterize binaural processing in the brainstem presents some difficulties, since the underlying assumption, that the ears are stimulated independently, may not be...
warranted at the frequencies of interest. Thus, although dichotic stimulation was needed to unmask and quantify binaural interactions in this study, free-field stimulation should provide a more physiologically relevant view of auditory nerve responses. With regard to cross talk between the ears, in earlier studies in frogs it was possible to reduce cross talk just by opening the mouth (Feng and Capranica 1976), but in the gecko this had little effect. To reduce cross talk in geckos, we inserted a mold that reduced the cross talk considerably (Fig. 10), although the introduced changes in middle-ear acoustics (Christensen-Dalsgaard 2005).

Comparisons with other directional hearing systems. In birds, ITD processing is mediated by a circuit in the nucleus laminaris, consistent with the Jeffress model, which assumes arrays of coincidence-detector neurons that respond maximally when phase-locked inputs converge from each ear simultaneously. Different conduction delays from each ear form “place” maps of sound location. Maps of ITD have been confirmed in barn owls, emus, and chickens, while data from the sister group to the birds, the crocodilians, are consistent with a place map of ITD (Carr and Konishi 1990; Overholt et al. 1992; MacLeod et al. 2006; Köppl and Carr 2008; Carr et al. 2009). In the mammalian equivalent of the nucleus laminaris, the medial superior olive, a place map has not been found (for review, see Joris and Yin 2007). Instead, studies of small mammals have revealed a tendency for the steepest region of the ITD tuning curve (its slope) to fall close to midline irrespective of best frequency (McAlpine et al. 2001; Brand et al. 2002; Pecka et al. 2008). McAlpine et al. (2001) have proposed that sound source location is instead represented by activity in two hemispheric spatial channels (Stecker et al. 2005).

Comparing birds and mammals, the individual neurons that make up maps of ITD in birds respond maximally to different preferred values, and their responses decrease if the actual stimulus either exceeds or falls short of the “best” value (Konishi 2003). In small mammals and lizards, sound source location can be computed from the overall discharge rate within the broadly tuned ITD channel on one side of the brain, provided that comparisons with the other ear allow resolution of ambiguity. The avian strategy uses delay lines to distribute the sensory parameter (location) across an array or map of neurons with different preferred parameter values, while the “two-channel” or rate coding strategy has no such requirement. The nature of delays in mammals appears multifaceted, while in the gecko, a fixed delay across the mouth cancels tympanic motion when it exactly compensates for the ITD presented through earphones or when the delay across the mouth exactly compensates for the sound location in free field. Thus, and with the possible exception of very low frequencies (see below), there should be no range of ITDs in gecko, such as recorded in chicken and barn owl (Wagner et al. 2007; Köppl and Carr 2008).

In frogs, the directional information in the auditory nerve at frequencies above ~400 Hz reflects the directionality of the tympanum, with up to 10-dB directional difference between ipsi- and contralateral stimulation (review in Christensen-Dalsgaard 2005; Schmitz et al. 1992; Ho and Narins 2006). The auditory nerve input is processed by binaural comparisons in the first auditory nucleus, the dorsal medullary nucleus (Christensen-Dalsgaard 2005). Both EI and excitatory-excitatory (EE) cells in the dorsal medullary nucleus have sharpened directionality (i.e., reduced contralateral sensitivity) compared with the auditory fibers (Christensen-Dalsgaard and Kanneworff 2005; Feng and Capranica 1976). The next station, the superior olivary nucleus, has a majority of EI cells (Feng and Capranica 1976). In the torus semicircularis, the directional information is effectively lateralized by cross-hemispheric inhibition (Melssen and Epping 1992), although there is no clear evidence for either separation of interaural time and level cues or for spatial maps of sound direction (review in Christensen-Dalsgaard 2005).
Directional neural processing has also been extensively studied in crickets and grasshoppers, two insect groups with acoustically coupled, directional ears (Stradner and Römer 2008; reviews in Pollack 1998; 2000). In both groups, the peripheral neural responses are directional, like in lizards, and primary interneurons like the cricket’s omega cell receive excitatory input from the ipsilateral ear and inhibitory input from the contralateral ear (Wohlers and Huber 1982). Similar processing might occur in the lizard central auditory system (see Fig. 2B for the hypothetical performance of a simple EI comparison in gecko). One major difference between cricket and lizard directionality, however, is that not all cricket or grasshopper neurons are directional, because their directional tuning is generally centered on the carrier frequency (Robert 2005).

**Frequency dependence of directionality.** Previous studies suggested that lizards with comparatively small heads and hence small ITDs should have limited ability to use binaural time cues (Szpir et al. 1990). However, the special construction of the lizard ear generates a much larger internal delay than the arrival-time delay at the eardrums. In the Tokay gecko (present data) and other lizards (Christensen-Dalsgaard and Manley 2008), delays are approximately three times the maximal arrival-time delay of 73 μs for a typical Tokay head width of 2.5 cm. What makes the lizard ear directionality especially acute is that this much larger measured delay matches the resonance frequency of the cavity and eardrum (Vossen et al. 2010). Frequencies (1,000–2,500 Hz) where the delay is about one-half wavelength are transmitted with high amplitude (unity gain) by the eardrum and middle ear cavity, leading to complete cancellation at some directions.

Laser vibrometry measures show that directionality is less at higher and lower frequencies. With decreasing best frequency, the phase difference between direct and indirect sound becomes smaller, i.e., constitutes a smaller proportion of the stimulus period, leading to relatively smaller amplitudes of eardrum motion (and thus a smaller change in firing rate) over the range of delays available to the gecko. At these low frequencies, however, temporal cues such as phase locking are available, and central detection of ITD could take place in, for example, the nucleus laminaris. Gecko phase locking is acute <500 Hz (Fig. 4) and they have a discernable nucleus laminaris (Yan et al. 2010). Above ~1 kHz, phase locking is degraded (Fig. 6) and thus will not be available at the frequencies where the ear is most directional (1–3 kHz). In this frequency range, however, the pressure gradient ear provides large changes in rate over the range of delays, as shown in this study. The directionality of the eardrums also decreases at high frequencies (>3 kHz; Figs. 1B and 3). An acoustical model of the gecko ear shows a strong eigen-value at 5 kHz in the smaller gecko *Hemidactylus*, indicating equal motion of the eardrums and loss of directionality (Vossen et al. 2010), and this eigen-value would presumably be lower in the larger Tokay gecko. Nevertheless, geckos hear well up to ≥5 kHz (Manley 1972; Köppl and Authier 1995; Werner et al. 2008; Brittan-Powell et al. 2010; Manley and Kraus 2010). Their ears may even be directional at high best frequencies because sound diffraction by the gecko head creates measurable ILDs (~5 dB at 4 kHz; Christensen-Dalsgaard and Manley 2005). Thus diffraction may supplement the cues generated by acoustical coupling at high frequencies.

**Neural processing of directional cues.** Lizards have two types of auditory nerve fibers, which differ both in peripheral features (Mulroy et al. 1974; Mulroy and Oblak 1985; Mulroy 1986) and electrophysiology (Weiss et al. 1974; Weiss et al. 1976). The auditory nerve fibers also have separate projection patterns into the brain (Barbas-Henry and Lohman 1988; Szpir et al. 1990; Yan et al. 2010). Tectorial fibers, which appear to correspond to mammalian type I fibers and avian cochlear nerve fibers, respond to lower best frequencies and project to both first order nuclei, an arrangement thought to have been present from the stem reptiles (Baird 1974; Miller 1980; Manley 1981; Carr and Code 2000). A second population of higher best frequency fibers evolved independently in lizards.
(Manley 2002), perhaps in connection with the emergence of tympanic hearing (Christensen-Dalsgaard and Carr 2008). These fibers project to a distinct medial region of the nucleus laminaris in the alligator lizard (Szpir et al. 1990). Despite this evidence for a distinct high frequency channel, we predict that comparisons across frequency would be useful in resolving ambiguity arising from changes in sound level, and could also resolve ambiguity in ITD cues (see Fig. 9C, where the 3 high best frequency examples all show phase ambiguity). On the other hand, an array of EI neurons, each tuned to different best frequencies, would generate independent, strongly lateralized responses and might simply be used in a “democratic” estimate of direction by the CNS. The term democratic was first used by Heiligenberg et al. (1978) to convey the idea that no unitary central representation is required; instead, correct responses are elicited to the extent that the majority of receptors are activated.

Whether the strong directionality of the lizard ear is reflected in different (simpler) central processing awaits further study. Binaural comparisons are still necessary, since any monaural directional response is ambiguous with respect to level and location. Since the eardrum directionality is strongly asymmetric across the midline (Fig. 2 A and C), lateralized responses could be generated by rate-based binaural comparisons, suggested by the simple model in Fig. 2B, and similar to the rate based comparisons in two lateralized channels proposed for the gerbil and guinea pig by McAlpine et al. (2001) and for the cat auditory cortex by Stecker et al. (2005). In the lizard, however, these responses could be generated as early as the lower brainstem. Robotic simulations show that simple binaural EI comparisons, like those observed in all vertebrates (Goldberg and Brown 1969; Feng and Capranica 1976; Edds-Walton and Fay 2003), plus bilateral motor excitation proportional to the EI-cell activity, produce an effective steering mechanism that the majority of receptors are activated.

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