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# The role of the chinchilla pinna and ear canal in electrophysiological measures of hearing thresholds

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Measurements of the acoustic transfer function (ATF) of the pinnae of 8 chinchillas were compared with the auditory-evoked potential (AEP) thresholds of 16 chinchillas measured in free field and with insert earphones. The ATF was measured in anesthetized chinchillas in a far-field condition in a semi-anechoic room using a logarithmic frequency sweep from 100 Hz to 20 kHz. Probe microphone measurements were collected with the probe opening at the tympanic membrane and in the same approximate position with the chinchilla removed from the sound field. For each animal's acoustic transfer function, the average of five in-the-ear and three free-field measurements were determined. The ATF exhibited a 5-dB passive gain at about 1 kHz and a broad resonance between 2.5 and 6 kHz of about a 10-dB gain. AEP thresholds were obtained from monaural chronically implanted chinchillas at 0.5, 1, 2, 4, and 8 kHz using first free-field and then insert earphone stimuli. The free-field sound pressure was measured with a microphone in the approximate position of the chinchilla's head. The earphone sound pressures were measured with a probe microphone positioned near the tympanic membrane. The free-field AEP hearing thresholds exhibited + 10-dB gain at 4 kHz compared to the insert earphone AEP thresholds. The agreement between the ATF and AEP derived transfer function suggested that the threshold differences at 4 kHz between the two testing configurations can be accounted for by the pinna and ear canal gain.
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## INTRODUCTION

Rosowski's analysis (Rosowski, 1991) of previous measurements of middle and external ear function demonstrates the importance of the acoustic properties of the pinna and ear canal on the sound collecting performance of the auditory periphery. He showed that the frequency dependence of the hearing threshold of the human, cat, and chinchilla could be predicted based on the known acoustic properties of the external and middle ear. He also showed the important role of the acoustic properties of the external and middle ear in producing the functional changes in the cochlea due to an acoustic trauma.

For the chinchilla, Rosowski utilized data from von Bismark's thesis (von Bismark, 1967; von Bismark and Pfeiffer, 1967). In his thesis, von Bismark measured the acoustic properties of the chinchilla external ear by cementing a probe-tube microphone into the bony portion of the ear canal, close to the tympanic membrane. Tone sweeps were played in the free field and measurements were taken from the microphone. Although the data have been referenced many times, they have never been published.

Behavioral audiograms indicate that the frequencies between 1 kHz and 6 kHz are the region of maximum sensitivity for the chinchilla (Fay, 1988; Heffner and Heffner, 1991). In humans, Hellström (1996) showed that the frequency of maximum temporary threshold shift (TTS) was correlated with the peak frequency of the acoustic transfer function (ATF) from the free-field to the tympanic membrane. The ATF was calculated as the ratio of pressures measured in the ear canal and measured in free field. The ATF characterized the acoustic response of the pinna and ear canal. Individuals with wide and long ear canals had more TTS due to a low-frequency noise exposure than people with a short and narrow ear canal.

The present study was conducted to replicate von Bismark's earlier data and to compare auditory-evoked potential (AEP) thresholds measured in free field and with insert earphones. An earlier version of these data was reported by Davis (1993) and Murphy and Davis (1996). By using a flexible probe-tube microphone similar to that used in humans (Hellström, 1996), surgery to the ear canal can be avoided and the animal can be used more than once. The probe-tube microphone permits *in situ* calibration of stimuli for evoked potential and otoacoustic emissions testing (Siegel and Hirohata, 1994; Whitehead *et al.*, 1995). The procedure reported here for measuring the ATF of chinchillas was similar to Hellström's technique used with humans.

In this paper, data from complementary experiments are presented. In Sec. I, direct measurements of the ATF are presented. In Sec. II, the estimates of the ATF are inferred through measuring the AEP thresholds using sound field and insert earphone configurations. Sections III and IV present a discussion of the comparison of the two experiments and conclusions, respectively.

## I. ACOUSTIC TRANSFER FUNCTION

### A. Methods

Four female and four male adult chinchillas with otoscopically verified normal external ears were subjects.[1] A logarithmic frequency sweep from 100 Hz to 20 kHz was generated by a Stanford Research SR780 dual channel network system analyzer. The sweep signal was amplified by a Brüel & Kjær 2706 power amplifier which drove a single element Bose 25 speaker. The signals from an Etymotic Research ER-7C probe tube microphone and a Brüel & Kjær 4165 1/2 in. microphone were measured simultaneously. The ATF was computed by the SR780 and stored on diskette for off-line analysis.

Five in-the-ear measurements and three free-field measurements were collected in an Eckel Industries AN-ECK-OIC semi-anechoic chamber. The chinchilla was placed on a square of stiff wire mesh attached to a floor stand approximately 115 cm from the front of the speaker. The animal's body was oriented along the axis of the speaker and was located at the same elevation as the speaker cone. A horizontal reference bar suspended above the speaker and the animal provided a spatial reference for positioning the probe microphone body and animal. During free-field measurements, the microphone body was suspended from the reference bar and the probe tip was positioned approximately where the chinchilla's tympanic membrane would be located.

To prevent head movement, subjects were administered ketamine (22 mg/kg im), xylazine (1.1 mg/kg im), and atropine sulfate (0.054 mg/kg im). The position of the pinna did not exhibit obvious changes in position due to the anesthesia. The flexible silicone probe tube of the ER-7C microphone was positioned in the ear canal with the probe tube opening approximately 1 mm from the tympanic membrane. The insertion was otoscopically inspected. The body of the ER-7C microphone was laid back over the animal's head. The microphone body did not alter the position of the pinna. Between each measurement, the probe microphone was removed from the ear canal, replaced and reinspected with an otoscope. The Brüel & Kjær 4165 microphone was positioned 80 cm from the front of the speaker and slightly below the chinchilla. The Brüel & Kjær microphone served as a reference microphone which was not moved or adjusted during the course of the measurements.

### B. Results

The data from the in-the-ear (ITE) and free-field (FF) measurements were averaged separately for each animal. The transfer function between in-the-ear to free-field measurements was calculated for each animal,

$$T_{\text{Animal,ITE-FF}}(f) = \frac{\overline{T_{\text{Animal,ITE}}(f)}}{\overline{T_{\text{Animal,FF}}(f)}}, \tag{1}$$

where $\overline{T_{\text{Animal,ITE}}(f)}$ was the average of the complex pressure ITE measurements and $\overline{T_{\text{Animal,FF}}(f)}$ was the average of the associated FF measurements. The complex transfer functions from the eight animals were then averaged to produce the overall acoustic transfer function between the ITE and FF measurements,

$$\overline{T_{\text{ITE-FF}}(f)} = \overline{T_{\text{Animal,ITE-FF}}(f)}. \tag{2}$$

Only one of the in-the-ear measurements for one of the animals proved to be considerably different from the rest of the data. The observer had noted in the data record book that the animal's head had rotated to the side giving cause to exclude that particular measurement. After the averages were computed, the dB magnitude was determined.

Figure 1 shows the average acoustic transfer function for the chinchillas (solid black line) and the range of the dB measurements (light gray shaded area). The transfer function exhibits approximately +3-dB gain from 700 Hz to 1.3 kHz, +10-dB gain from 2.5 to 6 kHz and −5-dB attenuation from 7 to 11 kHz. Table I lists the values of the acoustic transfer function and standard deviations at the standard audiometric frequencies. Above 15 kHz, the data exhibit considerable variability induced by the geometry of the pinna and ear canal. Tests in our laboratory of occluded and unoccluded ER-7 probe tubes show differences less than 10 dB for swept-sine stimuli above 14 kHz.

Von Bismark's results for type I and II transfer functions are compared with the averaged data from eight animals in

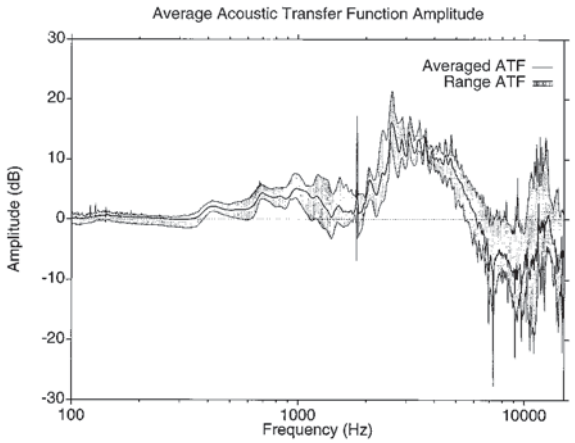


FIG. 1. The average acoustic transfer function and range (light gray) of the measurements collected from eight chinchillas. The transfer function exhibits a broad resonance between 2.5 and 6 kHz with about 10 dB of gain. From 700 Hz to about 1.3 kHz, a gain of about 4–5 dB is evident. From 7 to 10 kHz, an attenuation of about 5 dB is observed.

TABLE I. The average free field to eardrum acoustic transfer function and associated standard deviations at audiometric test frequencies measured from eight chinchillas.

| Frequency (Hz) | Transfer function ±standard deviation (dB) |
|---|---|
| 125 | +0.4±0.5 |
| 250 | +0.2±0.5 |
| 500 | +1.5±0.7 |
| 1000 | +5.1±1.3 |
| 2000 | +3.5±1.3 |
| 3000 | +10.6±1.4 |
| 4000 | +9.9±1.1 |
| 6000 | +1.3±2.4 |
| 8000 | −5.9±3.4 |

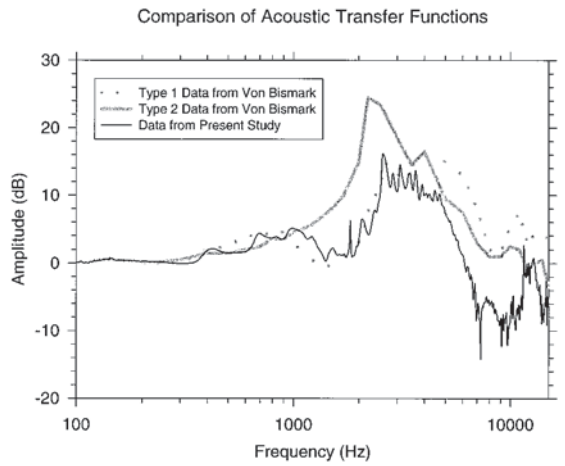


FIG. 2. Von Bismark's transfer functions contrasted with our average transfer function. Our data differs with the type I data primarily at the higher frequencies which could reflect the different placement of the microphone in the ear canal. The type II function is considerably wider and has a lower resonance peak. The pentobarbital anesthetic used by von Bismark was believed to compromise the middle ear ventilation in the type II data.

Fig. 2. Von Bismark attributed the type I data to animals which maintained normal middle ear ventilation. Differences between the present data and von Bismark's are evident at high frequencies and likely result from microphone placement. Whereas von Bismark cemented the microphone in the ear canal wall, placement here was in the inferior portion of the ear canal and the probe tip opening was directed towards the tympanic membrane.

The type II data in Fig. 2 exhibited more gain and a wider resonance. Von Bismark attributed the wider resonance in the type II data to the lack of ventilation of the middle ear due to the use of pentobarbital as the anesthetic agent which resulted in a static pressure difference across the tympanic membrane. A departure from normal quiescent middle ear pressure changes the compliance and middle ear transfer function. Since the ear canal is acoustically terminated with the middle ear, the pinna/ear canal transfer function will be affected by a poorly ventilated middle ear. Distortion product otoacoustic emission (DPOAE) data have shown that different anesthetic agents affect the ventilation of the middle ear of the mongolian gerbil (Zheng *et al.*, 1995). Ketamine did not depress the DPOAE response. However, injection of pentobarbital depressed DPOAE levels as did applying positive middle ear pressure. The ketamine/xylazine mixture used with other chinchillas in our lab has yielded similar ranges for AEP thresholds for awake and anesthetized animals as well as little difference in the DPOAE response. Normal middle ear ventilation was believed to have been maintained in the measurements taken here since they exhibit greater similarity with the type I data.

## II. AEP-DERIVED TRANSFER FUNCTION

### A. Methods

Sixteen adult chinchillas were monauralized via left cochlear destruction and implanted with chronic electrodes in the inferior colliculus and central sulcus region at SUNY-Buffalo Hearing Research Laboratories.[2] The animals were tested for auditory-evoked potentials in free-field conditions at SUNY-Buffalo and were then transported by automobile to NIOSH Taft Laboratories in Cincinnati. Following the quarantine period, the AEP hearing thresholds were measured using a Bio-Logic Navigator system equipped with insert earphones.[3]

The SUNY-Buffalo procedure has previously been described in Bancroft *et al.* (1991). The tone-burst stimuli (0.5, 1, 2, 4, 8, and 16 kHz) had a 5-ms rise/fall, 10-ms duration, and 100-ms interstimulus interval. Stimuli produced by a Loughborough TMS32020 16-bit digital to analog signal processing board were anti-alias filtered (20 kHz), attenuated and presented through Realistic 1218 speakers. The system was calibrated in SPL with a Larson-Davis 800B sound level meter and a Larson-Davis 2559 1/2-in. condenser microphone placed at the location of the chinchilla's head. Evoked responses from the chronic electrodes were amplified with a Grass P511 amplifier, digitized with a Loughborough TMS32020 16-bit A/D converter, averaged and analyzed. Animals were awake, alert, and restrained during testing. The restraint used by Buffalo had the animal facing the source with its nose pointing along the axis of the speaker, similar to the orientation for the ATF measurements.

The test system at NIOSH Taft Laboratories consisted of a Bio-Logic Navigator system, Etymotic Research ER-3 insert ear phones, ER-7C probe-tube microphone, and a Brüel & Kjær 2133 real-time frequency analyzer. The animals were awake, alert, and restrained during the test period (for a description of the restraint, see Snyder and Salvi, 1994). The restraint oriented the animal's head vertically rather than horizontally as Buffalo's restraint did. Once restrained, the probe-tube tip was positioned about 1 mm from the tympanic membrane. A neonatal foam plug was inserted while the probe tube was held against the pinna.[4] The ER-7C microphone was connected to the probe tube and the animal was placed in a small Industrial Acoustics Corporation sound-isolation booth. The active and reference electrodes were connected to the implant. A gold-cup electrode with electrode gel was connected to the left pinna which served as a ground.

The Bio-Logic system was programmed to collect a series of AEP responses at 5-dB intervals at 0.5, 1, 2, 4, and 8 kHz. The Bio-Logic system was not capable of producing a 16-kHz stimulus. Each response was the average of 512 presentations where the stimulus had a 2-ms rise/fall, 5-ms duration, and 43-ms interstimulus interval. The ear canal signal was calibrated in SPL before and after testing at the tone-burst frequencies using a constant 80 dB HL pure-tone signal which was detected by an ER-7C microphone and measured with the Brüel & Kjær 2133 real-time frequency analyzer. The average of the before and after calibration signal levels were used to correct the Bio-Logic HL measurements to SPL thresholds. One set of AEP thresholds was measured from each animal. AEP thresholds for both labs were determined at the level where an identifiable AEP response was no longer observed. If the AEP response was evident at 10 dB and not at 5 dB, the threshold was determined to be 7.5 dB.

TABLE II. The average free-field to insert earphone AEP derived transfer function, associated standard deviations and $t$ values measured from sixteen chinchillas. Data were tested with a two-tailed Student's $t$ test to identify which frequencies were significantly different from zero.

| Frequency (Hz) | Transfer function±standard deviation (dB) Insert earphone–free field (dB) | Significance $t$ values, $H_0$: $\mu=0$ |
|---|---|---|
| 500 | −1.5±4.9 | 0.1616 |
| 1000 | −4.5±4.7 | 0.0001* |
| 2000 | −1.1±5.3 | 0.3110 |
| 4000 | 9.6±6.8 | 0.0001* |
| 8000 | −2.0±9.3 | 0.3298 |

### B. Results

These measurements were performed primarily to provide a comparison between AEP data collected in the different configurations and also to determine whether any threshold shift occurred during transport between the two labs. The thresholds from the SUNY-Buffalo animals were as good or better than animals which have been implanted and tested at NIOSH. After completion of the exposures conducted at NIOSH (Davis *et al.*, 1996), the animals were returned to SUNY-Buffalo for further testing. Five of the 16 animals reported in this paper were in the Control group for the experimental study. The Control animals exhibited no positive threshold shifts, therefore differences between AEP thresholds were not attributable to transportation. Any differences are likely the result of different testing methods.

After determining the AEP thresholds, the differences between the free-field and insert earphone thresholds were calculated across frequency for all animals. A two-tailed Student's $t$ test was performed to identify those frequencies where the threshold difference was significantly different from 0-dB gain. The averaged differences for the various frequencies are listed in Table II along with their $t$ values. The prominent feature is a gain of 9.6 dB at 4 kHz. The differences at 1 kHz and at 4 kHz were statistically significant.

The statistical significance of the differences between AEP derived transfer function and the acoustic transfer function was tested with a Student's $t$ test (see Fig. 3). The AEP derived transfer function was significantly different from the ATF at 1 and 2 kHz ($|t|<0.05$). The two transfer functions were not significantly different at 0.5, 4, and 8 kHz. Thus the resonance at 4 kHz appears to result from the acoustical gain provided by the pinna and ear canal.

## III. DISCUSSION

From Fig. 2, data from this study agreed with von Bismark's type I data below 5 kHz. The resonance in the ATF of the chinchilla pinna significantly affected the level of sound reaching the middle ear and cochlea. In the 2.5–6 kHz range, the pinna and ear canal passively amplified the stimuli by as much as 20 dB for individual animals (see Fig. 1). From 8 to 10 kHz, the stimuli were attenuated by as much as 21 dB for individual animals.

Patterson and Hamernik (1992) have demonstrated increased hearing loss in chinchillas due to impulse noise as the carrier frequency of the impulse approached 1.5 kHz. The amount of hearing loss remained flat from 1.5 to 3.5 kHz, the highest carrier frequency used in their experiment. The small amount of gain seen in the acoustic transfer function between 700 and 1.3 kHz seems to agree with the increased hearing loss they found. The broad gain between 2.5 and 6 kHz would suggest that the region of greatest hearing loss would occur with carrier frequencies in that range. Their stimuli were presented at levels between 127 and 147 dB SPL and most certainly drove the cochlea beyond its mechanical limits (Spoendlin, 1976; Henderson *et al.*, 1991, 1994).

A similar study by Ahroon and Hamernik (1996) investigated the effect of changing the center frequency (0.5, 1, 2, 4, and 8 kHz) of a narrow-band impact presented at 115 dB SPL for either 6 h/day for 20 days or 24 h/day for 5 days. The loss of outer hair cells for these equal energy exposures was greatest for the 4-kHz exposure and next greatest for the 8-kHz exposure. When the energy of the impact at 1 kHz was varied from 109 to 127 dB (Hamernik and Ahroon, 1996), the loss of OHCs due to the 121-dB impact was comparable to the loss for 115-dB impacts at 4 kHz. These results suggest that the 10-dB effective gain for the pinna at 4 kHz enhanced the 115-dB impact. However, the OHC loss due to an 8-kHz 115-dB impact contradict what might be

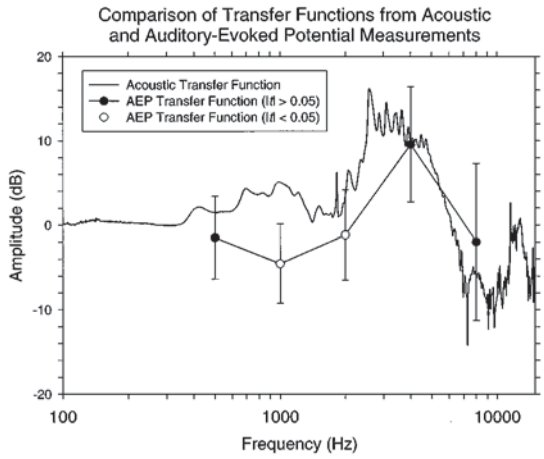


FIG. 3. A comparison of the acoustic transfer function and the auditory-evoked potential transfer function measured between free-field and in-the-ear canal conditions. The AEP and ATF functions agree at 500 Hz, 4 and 8 kHz, while differences at 1 and 2 kHz could be the result of terminating the ear canal with the ER-2 earplug. Error bars for the AEP derived transfer function represent the standard deviation of the measurements from 16 animals. Open symbols indicate that the AEP data are significantly different from the ATF data to a $p$ value $p<0.05$.

expected based upon the acoustic transfer function. Certainly, factors such as the pinna orientation and the location of the sound source relative to the head affect the resonant character of the auditory periphery. Shaw and Teranishi (1968) showed that as the elevation of a source increased, the resonant peak frequency for a blocked meatus condition moved to higher frequencies when examining the human pinna data. Additional data need to be collected to fully interpret hearing loss susceptibility in chinchillas based on the position of the source.

Microstructure regularly spaced at 260 Hz can be identified in the acoustic transfer function (see Fig. 1). These microstructure were produced by the room resonances and were observed in both the free-field and in-the-ear measurements. The use of two microphones, Brüel & Kjær and ER-7C, reduced the microstructure. Without constructing a better anechoic chamber, the microstructure are unavoidable. The resonance at 1 kHz is likely the result of the pinna given that the pinna is the larger structure and would have a lower resonant frequency. However, the AEP transfer function exhibits a dip at 1 kHz.

Several possible explanations might account for the difference between ATF and AEP transfer functions. One explanation might be the orientation of the animal in the testing environment during free-field stimulation. The orientation of the pinna during awake AEP testing could be slightly different than the anesthetized position for ATF measurements. The effect of pinna orientation has been observed in AEP tests of chinchillas in free-field conditions (Davis *et al.*, 1996). In the raw data of that experiment (Davis *et al.*, 1996), an improved threshold (5–10 dB at 8 kHz) in *nonexposed control* animals resulted from experimental procedures. The difference was the effect of propping or not propping the pinna toward the speaker during testing. Because the pinna effects were observed at 8 kHz and not 1 kHz, the orientation of the animal probably is *not* responsible for the differences observed between ATF and AEP derived transfer functions.

A second possibility might be the different stimulus envelope parameters used to determine AEP thresholds in free field and insert earphone configurations. Additional testing of chinchillas using the envelope parameters yielded no significant effect on the AEP thresholds.

A third possible explanation would be a difference between the calibration of the test delivery systems between SUNY-Buffalo and NIOSH. The sound level calibrators at NIOSH have NIST traceable calibrations and are used to calibrate the Brüel & Kjær microphone and ER-7C microphone in the free-field condition. Daily calibrations of the ER-7C microphone during AEP testing are performed using the 94-dB signal provided by the preamplifier on the ER-7C. The microphones at Buffalo are calibrated with a NIST traceable calibrator and the free-field delivery system is checked regularly to ensure it is properly calibrated.

## IV. CONCLUSIONS

The 10-dB gain at 4 kHz in the AEP data can be attributed to the effective gain of the pinna and ear canal. The attenuation at 8 kHz exhibits considerably more variation in the AEP data than in the ATF data. The placement of the probe tip for the AEP measurements was somewhat more difficult since the animals were not anesthetized and an insert earphone prohibited inspection of the final placement. Thus the data permit comparison of chinchilla hearing thresholds between free field and insert earphone configurations for frequencies below 6 kHz. The effects of the pinna orientation and the probe tube placement will complicate comparisons above 6 kHz.

The present study confirms von Bismark's earlier measurements and demonstrates the utility of using a flexible probe-tube microphone to measure the acoustic transfer function in small animals. As well, the passive amplification of the pinna produces significant effects that must be considered when when determining the transformation of free-field measurements of stimuli to eardrum sound pressure levels.

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[1]Acoustic transfer function measurements were approved by the NIOSH Division of Biomedical and Behavioral Science (DBBS), Animal Care and Use Committee (ACUC), protocol C69DAV.

[2]Surgical procedures were approved by the SUNY-Buffalo ACUC, protocol COM05080N.

[3]SUNY-Buffalo animals were transported to NIOSH Taft Laboratories and measurements performed on them were approved by the NIOSH DBBS ACUC, protocol C73DAV.

[4]Although the chinchillas had been monauralized, a foam insert earphone was inserted in the contralateral ear canal. The standard chronic electrode implant preparation at NIOSH Taft Laboratories does not include monauralization and therefore AEP tests are performed with insert earphones to isolate the response. The neonatal (tan) foam plugs provide an excellent fit for chinchillas and yield $>60$ dB of isolation from acoustic cross talk.

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