Correlations between noninvasive and direct physiological metrics of auditory function in chinchillas with noise-induced hearing loss

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Noninvasive physiological tools for assessing auditory function in humans can provide valuable information when behavioral tests are not possible. Furthermore, these tools hold promise to provide greater insight into underlying cochlear pathologies. In this study, we used noninvasive distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) to estimate changes in auditory function in chinchillas with noise-induced hearing loss. Two aspects of cochlear function, sensitivity (threshold) and frequency selectivity (tuning), were measured directly using neurophysiological recordings from auditory-nerve (AN) fibers to assess the predictive value of DPOAEs and ABRs. Both DPOAE amplitude and ABR threshold were well correlated (R-square ~0.5) with AN fiber threshold near stimulus frequency. For DPOAEs, the correlation was strongest for cochlear function near F2. Correlations of both noninvasive metrics with AN tuning were weaker but statistically significant. The relatively weak correlation between DPOAE amplitude and AN tuning was unexpected because both measures are tied to the integrity of outer hair cells. Alternatively, previous results suggest ABR latency may be more predictive of AN tuning. Ultimately, DPOAEs may be a more practical clinical and research screening tool for hearing loss than ABRs due to shorter recording time. Research supported by NIH (NIDCD) F32-DC012236 and R01-DC009838.

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INTRODUCTION

Otoacoustic emissions (OAEs) recorded in the ear canal have attracted considerable research attention as a noninvasive measure of auditory function since their discovery by Kemp (1978). In humans, OAEs evoked by paired tone stimuli, known as distortion-product OAEs (DPOAEs), hold promise to provide information about the status of the cochlea in situations when behavioral assessment of hearing performance is not possible (e.g. in infants and young children). So far, the predictive power of DPOAEs in the clinic has been relatively modest, due at least in part to the complex interaction of cochlear sources and mechanisms by which DPOAEs are generated (Shaffer et al., 2003). DPOAEs also hold promise to provide greater detail regarding underlying cochlear pathology. Specifically, DPOAE measurements should closely relate to outer hair cell (OHC) function because current data strongly implicate the OHCs in OAE generation over other components of cochlear transduction such as the inner hair cells (IHCs).

A number of previous studies in animals have examined the ability of DPOAEs and other noninvasive measures of auditory function such as the auditory brainstem responses (ABR) to predict histopathological damage in individuals with hearing loss due to acoustic overexposure and ototoxic drugs. ABRs are neural responses recorded from the scalp surface that reflect the net electrical activity of neural centers throughout the auditory system. In general, these studies show that DPOAE measurements are moderately correlated with histopathological damage to OHCs (Trautwein et al., 1996; Hofstetter et al., 1997; Davis et al., 2004) while ABR measurements are more closely tied to IHC damage and loss of cochlear neurons (e.g. Harding et al., 2002).

Relatively less work has examined relationships between noninvasive metrics of auditory function and direct measurements of cochlear sensitivity (threshold) and frequency selectivity (tuning bandwidth) made at the level of individual auditory-nerve (AN) fibers. Ngan and May (2001) and Henry et al. (2011) examined relationships between ABR measurements and AN response characteristics. Here, we extend this work by focusing on the ability of DPOAE amplitude to predict AN fiber threshold and frequency tuning in chinchillas subjected to acoustic overexposure. In general, we expected DPOAE amplitude to be strongly correlated with AN frequency tuning because both measures are closely tied to OHC function (Liberman and Dodds, 1984). A weaker correlation of DPOAE amplitude with AN threshold was expected because DPOAE amplitude is related primarily to OHC function (Trautwein et al., 1996) while AN threshold is related to the function of both OHCs and IHCs (Liberman and Dodds, 1984). As a final goal, we compared the relative abilities of DPOAEs and ABRs to predict cochlear function.

METHODS

DPOAEs, ABRs, and AN fiber responses were recorded from 23 chinchillas weighing 0.4-0.6 kg. We induced hearing loss in 20 of the animals by overexposure to noise, while the remaining 3 animals served as normal-hearing controls. DPOAEs and ABRs were recorded both before and after induction of hearing loss in 15 of the 20 overexposed animals and after induction only in the remaining 5 overexposed animals. In noise-overexposed animals, post-exposure recordings of DPOAEs and ABRs were made 4 or more weeks after the noise overexposure and were followed immediately by AN fiber recordings. In normal-hearing control animals, a single session of DPOAE and ABR recordings was followed immediately by AN fiber recordings. In total, AN responses were recorded from 128 control fibers and 440 noise-overexposed fibers (mean: 25.8 fibers per animal; range 5-59 fibers per animal). All procedures were conducted with the approval of the Purdue Animal Care and Use Committee.

Noise-overexposure

Animals were anesthetized with xylazine (1-1.5 mg/kg subcutaneous) followed by ketamine (50-60 mg/kg intraperitoneal). Atropine (0.1 mg/kg; intramuscular) was given to control mucous secretions and eye ointment was applied to prevent drying of the eyes. We induced hearing loss by overexposing animals to a 116 dB SPL half octave band of noise with a center frequency of 500 Hz for two hours. Noise was presented through an enclosed woofer (Selenium 10PW3) positioned 25 cm above the animal’s head in a sound attenuating chamber (Industrial Acoustics Company, Bronx, NY). The SPL of the noise was calibrated at the ear with a type 2 sound level meter (Simpson 886-2, Elgin, IL). Anesthesia was maintained with supplemental ketamine injections (20-30 mg/kg...
intraperitoneal). Body temperature was maintained at 37°C with a feedback controlled heating pad (Harvard Apparatus 50-7220F).

Noninvasive DPOAE and ABR Recordings

Animals were anesthetized with xylazine (1-1.5 mg/kg subcutaneous) followed by ketamine (50-60 mg/kg intraperitoneal). Atropine (0.1 mg/kg intramuscular) was given to control mucous secretions and eye ointment was applied. A sealed microphone (Etymotic ER-10B) and pair of transducers (Etymotic ER-2) were inserted into the right ear canal to record DPOAEs and present acoustic stimuli, respectively.

For DPOAEs, paired tone stimulus frequencies \( f_1 \) and \( f_2 \) were presented with a frequency ratio \( f_2/f_1 \) of 1.2. Twenty-seven stimulus combinations were presented in succession with \( f_2 \) values increasing in logarithmic steps from 500 Hz to 12 kHz. For each combination, tone stimuli \( f_1 \) and \( f_2 \) were presented over 5 repetitions by separate transducers at a level of 75 dB SPL. Stimuli were 1 second in duration with a 1-second silent inter-stimulus interval. DPOAE recordings were analyzed in the frequency domain to determine the SPL of the emission at the distortion product frequency \( 2f_1-f_2 \).

ABRs were recorded in response to 5-ms tone bursts of decreasing SPL. Tone bursts were presented with 0.5-ms onset and offset ramps and alternating polarity at a rate of 20 stimuli per second. Responses were recorded using subdermal needle electrodes inserted at the dorsal midline between the eyes (non-inverting), posterior to the right pinna (inverting), and the bridge of the nose (common ground). ABRs were amplified 20,000 times (World Precision Instruments model ISO-80; Dagan model 2400A) and band-pass filtered from 0.3 to 3 kHz (Krohn-Hite model 3550 filter). Each ABR waveform saved for analysis was the average of 1000 individual responses to stimuli of the same frequency and intensity. ABR thresholds at 0.5, 1, 2, 4, and 8 kHz were determined using a previously described cross-correlation procedure (Henry et al. 2011).

AN Fiber Recordings

The procedure used to access the AN and record single-fiber responses has been described previously (Kale and Heinz, 2010). In short, animals were anesthetized with xylazine (1-1.5 mg/kg subcutaneous) followed by ketamine (50-60 mg/kg intraperitoneal) and placed in a stereotaxic device in a sound attenuating booth. Subsequent anesthesia was maintained with sodium pentobarbital (≈7.5 mg/kg/h intravenous). Physiological saline (2-5 ml/h intravenous) and lactated ringers (20-30 ml/24 h subcutaneous) were also given, and a tracheotomy performed to facilitate breathing. Body temperature was maintained at 37°C with a feedback controlled heating pad (Harvard Apparatus 50-7220F). The skin and muscles overlying the skull were transected to expose the ear canals and bullae. The ear canals were dissected and hollow ear bars inserted. The right bulla was vented through 30 cm of polyethylene tubing. A craniotomy was opened in the posterior fossa, and the cerebellum partially aspirated and retracted medially to expose the cochlear nucleus and medial trunk of the AN.

Acoustic stimuli were presented through a dynamic loudspeaker (Beyerdynamic model DT-48, Farmingdale, NY, USA) sealed to the right ear bar, and calibrated using a probe tube microphone (Etymotic model ER-7C) placed within a few mm of the tympanic membrane. AN activity was recorded using a 10-30 MΩ glass microelectrode advanced into the AN by a hydraulic micro drive (Kopf model 640, Tujunga, CA, USA). The electrode signal was amplified (Dagan model 2400A) and band pass filtered from 0.02 to 6 kHz (Krohn-Hite model 3550). Action potentials were identified using a time amplitude window discriminator (Bak Electronics), and their timing recorded with 10 µs resolution.

Single fibers were isolated by listening for action potentials while advancing the electrode through the medial trunk of the AN and playing a broadband noise search stimulus. For each fiber encountered, characteristic frequency (CF), threshold at CF, and tuning bandwidth (measured 10 dB above threshold) were determined using an automated tuning curve algorithm that tracked the minimum sound level required for a 50-ms tone to evoke at least one more action potential than a subsequent 50-ms silent period (Chintanpalli and Heinz, 2007). For fibers from noise-overexposed animals with broad frequency tuning, CF was chosen based on the steep high frequency slope of the tuning curve because this provides a robust estimate of pre-exposure CF (Liberman, 1984).
Analysis

We analyzed the relationships between noninvasive metrics of auditory function and direct measurements of cochlear sensitivity and frequency selectivity based on AN responses using regression analyses of normalized variables. Frequency-normalized metrics of DPOAE amplitude and ABR threshold were calculated as the observed DPOAE amplitude minus the mean DPOAE amplitude of control animals at the same stimulus frequencies and the observed ABR threshold minus the mean ABR threshold of control animals at the same stimulus frequency, respectively. A CF-normalized metric of cochlear sensitivity was calculated as the observed AN threshold minus the mean threshold of a large population of normal hearing control fibers at the fiber’s CF (i.e. from Kale and Heinz, 2010). Similarly, CF-normalized frequency selectivity was calculated as the octave difference between the observed tuning-curve bandwidth and the mean bandwidth of the normal hearing population at the fiber’s CF. These normalized variables correspond to vertical distances between individual observations and normal-hearing trend lines in Fig. 1.

**FIGURE 1.** Physiological data collected from chinchillas with normal hearing and hearing loss due to noise-overexposure. (a) DPOAE amplitude as a function of stimulus frequency ($f_2$), (b) AN fiber threshold as a function of CF, and (c) AN fiber frequency tuning-curve bandwidth as a function of CF. Solid black lines are trend lines for normal-hearing data.
RESULTS AND DISCUSSION

Noninvasive DPOAE and ABR measurements

DPOAE amplitude was relatively consistent across normal-hearing control animals and exhibited slight maxima at 2 and 8 kHz (Fig. 1a). Noise-overexposure generally caused a reduction in DPOAE amplitude, but to varying degrees in different individual animals. Some animals showed no apparent reduction in amplitude while others showed up to 30 dB of reduction. Similar variability was observed in ABR thresholds (data not shown) and AN fiber response characteristics (described below) in response to the acoustic overexposure, consistent with the idea that some individuals have “tougher” ears that are more resistant to physical damage in the presence of loud sound (e.g. Cody and Robertson, 1983).

Cochlear Sensitivity and Frequency Selectivity

AN fibers of normal-hearing control animals generally had thresholds within 15 dB of 20 dB SPL across the CF range studied (Fig. 1B). The bandwidth of frequency tuning in control fibers increased with increasing CF (Fig. 1C). Noise-overexposure generally caused increases in both the threshold and bandwidth of frequency tuning of AN fibers. Threshold elevation occurred across the CF range while increases in tuning bandwidth were most prominent in neurons with higher CFs. Similar to the noninvasive auditory measurements, threshold and tuning bandwidth varied considerably across noise-overexposed AN fibers from different animals. Some fibers had threshold and frequency tuning similar to controls while others showed up to 50 dB of threshold elevation and a four-fold increase in tuning bandwidth.

Correlations between Noninvasive and Direct Measures of Auditory Function

Regression analyses of normalized variables indicated a negative correlation between DPOAE amplitude and AN threshold at the cochlear place tuned to $f_2$ (R-square=0.486, P<0.001; Fig. 2A), while ABR threshold was positively correlated to AN threshold at the cochlear place tuned to the stimulus frequency (R-square=0.454, P<0.001; Fig. 2B). Both noninvasive metrics appear to have similar, moderate abilities to predict cochlear sensitivity in chinchillas with varying degrees of noise-induced hearing loss. Correlations between DPOAE amplitude and AN threshold at cochlear places tuned to $2f_1-f_2$ and $f_1$ were generally weaker, consistent with current theoretical models of DPOAE generation (Shaffer et al., 2003).

DPOAE amplitude was negatively correlated with the bandwidth of cochlear frequency tuning at the $f_2$ location within the cochlea (R-square=0.281, P<0.001; Fig. 2C). In contrast to previous predictions however, the R-square value for the relationship with AN frequency tuning was substantially lower than the R-square value for the relationship with AN threshold (see above). The lower R-square value is surprising because both DPOAE amplitude and cochlear frequency tuning are thought to rely on a common physiological mechanism linked to OHC function (Liberman and Dodds, 1984; Trautwein et al. 1996). Finally, a weak positive relationship was observed between ABR threshold and cochlear frequency tuning (R-square=0.199, P<0.001; data not shown). Previous limited work suggesting that ABR latency is predictive of AN frequency tuning should be expanded on to determine whether DPOAE amplitude or ABR latency provide a better estimates of cochlear frequency tuning (Henry et al. 2011).

Conclusions

In conclusion, we found that both DPOAE amplitude and ABR threshold are moderate predictors of cochlear sensitivity in chinchillas with noise-induced hearing loss. In general, the relative abilities of these metrics to predict cochlear sensitivity seem similar to their abilities to predict histopathological damage to IHCs and OHCs (e.g. Davis et al., 2004; Hofstetter et al. 1997). Ultimately, DPOAEs may be a more useful screening tool in clinical and research settings because they are less time consuming to record than ABRs and require fewer specialized procedures and materials.
FIGURE 2. (a) Normalized AN fiber threshold plotted as a function of normalized DPOAE amplitude in the same animal at the same frequency (i.e. CF=\(f_2\)), (b) normalized AN fiber threshold plotted as a function of normalized ABR threshold in the same animal at the same frequency, and (c) normalized AN fiber frequency tuning plotted as a function of normalized DPOAE amplitude in the same animal at the same frequency (i.e. CF=\(f_2\)). The trend lines are fits of the linear regression models.

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