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Relation of focal hair-cell lesions to noise-exposure parameters from a 4- or a 0.5-kHz octave band of noise

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ABSTRACT

In a previous study, we examined the relation between total energy in a noise exposure and the percentage losses of outer (OHC) and inner (IHC) hair cells in the basal and apical halves of 607 chinchilla cochleae [Harding, G.W., Bohne, B.A., 2004a. Noise-induced hair-cell loss and total exposure energy: analysis of a large data set. J. Acoust. Soc. Am. 115, 2207–2220]. The animals had been exposed continuously to either a 4-kHz octave band of noise (OBN) at 47–108 dB SPL for 0.5 h–36 d, or a 0.5-kHz OBN at 65–128 dB SPL for 3.5 h–433 d. Interrupted exposures were also employed with both OBNs. Post-exposure recovery times ranged from 0 to 913 days. Cluster analysis was used to separate the data into three magnitudes of damage. The data were also separated into recovery times of 0 days (acute) and >0 days (chronic) and the apical and basal halves of the organ of Corti (OC). A substantial part of these hair-cell losses occurred in focal lesions (i.e., $\geqslant$50% loss of IHCs, OHCs or both over a distance of $\geqslant$0.03 mm). This aspect of the damage from noise was not included in the previous analysis. The present analysis describes, within the same three clusters, the apex-to-base distribution of 1820 focal lesions found in 468 of 660 (71%) noise-exposed cochleae. In these cochleae, OC length in mm was converted to percent distance from the apex. The lesion data were analyzed for location in percent distance from the apex and size (mm) of the lesions. In 55 of 140 (39%) non-noise-exposed, control OCs, there were 186 focal hair-cell lesions, the characteristics of which were also determined. Focal lesions with hair-cell loss $\geqslant$50% involved predominantly OHCs, IHCs only, or both OHCs and IHCs (i.e., combined OHC–IHC lesions). The predominantly OHC and combined lesions were pooled together for the analysis. The distributions of lesion location (in percent distance from the apex), weighted by lesion size (in percent of OC length) were tallied in 2%-distance bins. In controls, focal lesions were uniformly distributed from apex to base and 70% of them were pure IHC lesions. In cochleae exposed to the 4-kHz OBN, lesions were distributed throughout the basal half of the OC. In cochleae exposed to the 0.5-kHz OBN, lesions occurred in both halves of the OC. With continuous exposures, 74% of the lesions were predominantly OHC or combined lesions. With interrupted exposures, 52% of the lesions were OHC or combined lesions. Lesion size was generally larger in the chronic compared to acute cochleae with similar exposures. There was a minimum total energy at which focal lesions began to appear and slightly higher energies resulted in nearly all exposed cochleae having focal lesions.

1. Introduction

Noise-induced loss of hair cells in the cochlea may be scattered or concentrated. In the low-frequency region [i.e., apical half of the organ of Corti (OC)], beginning noise damage appears as loss of outer hair cells (OHC) scattered over a broad extent of the OC. In the high-frequency region (i.e., basal half of the OC), beginning noise damage often takes the form of small, concentrated losses of sensory cells. Because of these very different patterns of damage, we summarize hair-cell loss in two ways: (1) when cell loss is scattered, the average percentage of missing hair cells is calculated over a specific percentage length of the OC (e.g., 50%); (2) when hair-cell loss is concentrated, the length (in mm) is measured and the type(s) of affected cells determined. We deem the damage as a concentrated lesion when inner-hair-cell (IHC) or OHC loss is $\geqslant$50% over a distance of at least 0.03 mm (i.e., distance encompassed by three IHCs or four OHCs). Furthermore, concentrated lesions are classified as being predominantly OHC, pure IHC, or

Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; DPOAE, distortion product otoacoustic emission; IHC, inner hair cell; K–W, Kruskal–Wallis; OBN, octave band of noise; OC, organ of Corti; OC-Wipeout, region of complete loss of the OC; OHC, outer hair cell; OHC+, OHC, combined OHC–IHC, and OC-Wipeout focal lesions; PLS, permanent level shift (DPOAE); PTS, permanent threshold shift (ABR); ROS, reactive oxygen species; SPL, sound pressure level.

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combined OHC–IHC, depending on which hair-cell type(s) exceed 50% loss (Bohne et al., 1990).

Although seen in earlier publications (e.g., Bredberg,1968; Hunter-Duvar and Bredberg, 1974), we initially coined the term 'focal hair-cell lesions' in reference to concentrated hair-cell loss based on our findings in noise-exposed chinchilla cochleae (Clark and Bohne, 1978; Bohne and Clark, 1982). Inspection of published photomicrographs and cytocochleograms reveals that focal hair-cell lesions occur in human cochleae (e.g., Bredberg, 1968, 1973; Hawkins and Johnsson, 1976; Johnsson, 1974; McGill and Schuknecht, 1976) and the cochleae of other animals commonly used for noise research, including cats (e.g., Liberman and Kiang, 1978; Liberman and Mulroy, 1982), mice (e.g., Ou et al., 2000a,b; Harding et al., 2005) and guinea pigs (e.g., Stockwell et al., 1969; Thorne and Gavin, 1985; Fredelius, 1988).

An octave band of noise (OBN) with a center frequency of 4 kHz (i.e., high frequency for chinchillas and humans) damages the high-frequency region of the OC only (Bohne, 1976; Salvi et al., 1982; Bohne and Harding, 2000), unless the SPL is very high (e.g., $\geqslant\sim$108 dB; Kim et al., 1997; Harding et al., 2002). In the chinchilla, most of the OHC loss and essentially all of the IHC loss occur after exposure termination (Harding and Bohne, 2004a). Most hair-cell loss from this exposure occurs one-half an octave above the center of the OBN (i.e., $\sim$6 kHz). However, focal lesions occur up to 2 octaves above and 1 octave below the band (Harding and Bohne, 2007). Some hair-cell loss is scattered around the 4-kHz region while some loss is concentrated in one or more focal lesions.

A 0.5-kHz OBN (i.e., low frequency for chinchillas and humans) initially injures the low-frequency region of the OC. In chinchillas, most damage in the low-frequency region involves OHC loss scattered over a broad area (Bohne, 1976; Bohne and Clark, 1982; Salvi et al., 1982). Most of this loss occurs during the exposure (Harding and Bohne, 2004a). Substantial IHC loss is rare in the apical half of the OC (e.g., Bredberg, 1968; Bohne and Clark, 1982). Focal OHC lesions occur in the apex, but they are fewer in number and shorter in length. About half of the cochleae exposed to the 0.5-kHz OBN for 24 h or more also sustain damage in the high-frequency region of the OC. This loss in the basal half of the OC usually consists of focal hair-cell lesions that are morphologically indistinguishable from those caused by the 4-kHz OBN exposure (Bohne, 1976; Bohne and Harding, 2000).

Focal lesions are important because even a small lesion involving IHCs and associated afferent-nerve-fiber loss produces a permanent threshold shift (PTS) at the frequency-place of the lesion (e.g., Nordmann et al., 2000). Concentrated OHC loss of sufficient length will also produce a PTS (e.g., Ahmad et al., 2003). Isolated focal lesions involving only OHCs that are relatively small (i.e., <1 mm) do not produce a PTS (e.g., Harding and Bohne, 2004b).

Previously, we examined the relation between total energy in a noise exposure and total percentage losses of OHCs and IHCs in the basal and apical halves of 607 chinchilla cochleae (Harding and Bohne, 2004a). Most of the animals had been exposed continuously to either a 4-kHz OBN at 47–108 dB SPL for 0.5 h–36 d, or to a 0.5-kHz OBN at 65–128 dB SPL for 3.5 h–433 d. For both OBNs, interrupted exposures were also employed in some animals. Post-exposure recovery times ranged from 0 to 913 days. In chinchillas, exposures at 47–95 dB SPL for 1–9 days are considered moderate-level, moderate-duration. Exposures at 108–128 dB SPL for a few hours are considered high-level, short-duration. Cluster analysis was used to separate the data into three magnitudes of damage. The data were also separated into recovery times of 0 days (i.e., acute) and >0 days (i.e., chronic). It was found that moderate-level, moderate-duration exposures produced OHC and IHC losses that were related to total energy, while hair-cell losses from high-level, short-duration exposures were not related to total energy. In addition, most OHC loss occurred after the 4-kHz OBN was over, while most OHC loss with the 0.5-kHz OBN occurred during the exposure. With both OBNs, most IHC loss occurred during the exposure. A substantial fraction of hair-cell losses in these cochleae occurred in focal lesions. This aspect of the damage caused by the noise exposure was not included in the Harding and Bohne (2004a) analysis. Previously, Harding and Bohne (2007) examined one aspect of the focal-lesion issue. A data set was assembled from our collection that included only focal lesions <1.5 mm in size in cochleae exposed for <10 days with no recovery or very short recovery times. It was found that these focal lesions were widely distributed, well above and below the OBN. The objective of that analysis was to determine if previously undetected spikes outside the exposure band and/or harmonics and distortion products could explain the creation of focal hair-cell lesions. It was found that noise spikes, harmonics, and distortion products could not account for the location of focal lesions. The present report describes, within three clusters as was done in Harding and Bohne (2004a), an analysis of focal-lesion location, size, and apex-to-base distribution in all cochleae exposed to the 4- or 0.5-kHz OBN, regardless of exposure duration and recovery time, including cochleae processed and analyzed since our earlier reports.

2. Methods

2.1. Animals and noise exposures

Chinchillas, 1–3-y-old of either sex, were exposed free field to either a 4-kHz or a 0.5-kHz OBN at a variety of levels and durations (i.e., 47–128 dB SPL, 0.5 h–432 d, respectively) as shown in Table 1. For the present analysis, high-level for the 4-kHz OBN was considered to be 108 dB SPL. For the 0.5-kHz OBN, high level was greater than or equal to 120 dB SPL. Short-duration was considered to be 0.5–24 h and moderate duration 1–36 d for continuous exposures. A few of the chronic animals were exposed to the 0.5-kHz OBN at 95 dB SPL for longer durations (i.e., 27 of 77 animals {35%}; 45–432 d). Data from these animals were included because it was likely that much of the hair-cell loss occurred fairly early in the exposure. For interrupted exposures, moderate duration was considered to be 9–252 d, depending on total interruption time. Because the chinchilla's life span is 15–20 years, age-related hair-cell loss was minimal in our animals.

2.2. Tissue processing and evaluation

The cochleae from 419 noise-exposed and 118 non-noise-exposed animals were available for histopathological examination. This sample included all completely processed and analyzed cochleae in our collection which had been exposed to either of the two OBNs. Both cochleae were analyzed from 241 noise-exposed animals and 22 controls; one was analyzed in the other animals. The continuously exposed cochleae were processed immediately post-exposure (acute group) or after recovery times ranging from 1 to 913 days (chronic group). The animals exposed on interrupted schedules (e.g., 6 h/d, 6 h/2d, 45 min on – 15 min off) for 9–252 days had recovery times ranging from 0 to 365 days (interrupted group). Due to longer exposure times and periodic recovery times, the interrupted group was considered to be comparable to the chronic group. The details for the histological processing protocol can be found in Bohne (1972). Briefly, the cochleae were fixed in vivo with a buffered solution of osmium tetroxide, dehydrated, embedded in plastic, and dissected into flat preparations. Organ of Corti length was measured along the junction of the pillar-cell heads as described in Ahmad et al. (2003) and missing OHCs and IHCs were counted using phase-contrast microscopy at 625 or 1250$\times$ magnification as described in Bohne and Clark (1982). The results were expressed in percent missing as a function of percent