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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

Table 1
Number of animals by OBN, exposure level, duration, and recovery group.

	Acute		Chronic		Interrupted	
	Duration	# Animals	Duration	# Animals	Duration	# Animals
4-kHz OBN dB[a] (SPL)						
47–72	2–9 d	9	2–9 d	7		–
80	2–9 d	5	1–9 d	7	36–252 d	12
86	1–9 d	14	1–36 d	20	9–72 d	14
92	1 d	4	1 d	3		–
108	0.5–24 h	35	1–24 h	86	9 d	2
Total		67		123		28
0.5-kHz OBN dB (SPL)						
65–85	2–9 d	9	2–9 d	6		–
95	2–9 d	31	1–432 d	77	36–252 d	47
108	2–9 d	2	9 d	1	9–36 d	4
120–128	3.5–14 h	4	3.5–13 h	20		–
Total		46		104		51

[a] Not including 16 dB increase in SPL at eardrum due to ear-canal resonance.

distance from the apex of the OC. The total percent missing was then tallied for the basal and apical halves (i.e., ⩾50%- and <50%-distance from the apex, respectively) of the OC.

2.3. Focal lesions

Focal lesions, if any, were identified in each OC, their size measured in mm, and their location and extent expressed as a function of percent distance from the apex. Focal lesions were classified as: OHC (Fig. 1 A, OHC loss ⩾50%; IHC loss <50%); IHC (Fig. 1B, IHC loss ⩾50%; OHC loss <50%); or combined OHC–IHC (Fig. 1C, both OHC and IHC loss ⩾50%). Many OHC lesions had no IHC loss while others had small losses. Most of the IHC lesions involved no OHC loss (i.e., pure IHC lesions). The basal/apical extent of OHC loss in combined OHC–IHC lesions was almost always greater than that for IHC loss. In some cases, an entire region of the OC was missing, including all supporting cells. This is termed an 'OC-Wipeout' (Fig. 1D). In an OC-Wipeout, the basilar membrane and the tunnel at the edges of the OC-Wipeout were covered by a thin layer of squamous epithelium. There was almost always considerable OHC loss basal and apical to an OC-Wipeout.

The distribution, size and type of 2006 focal lesions in 140 non-noise-exposed and 660 noise-exposed cochleae (Table 2) were analyzed. These specimens included cochleae from 118 controls and

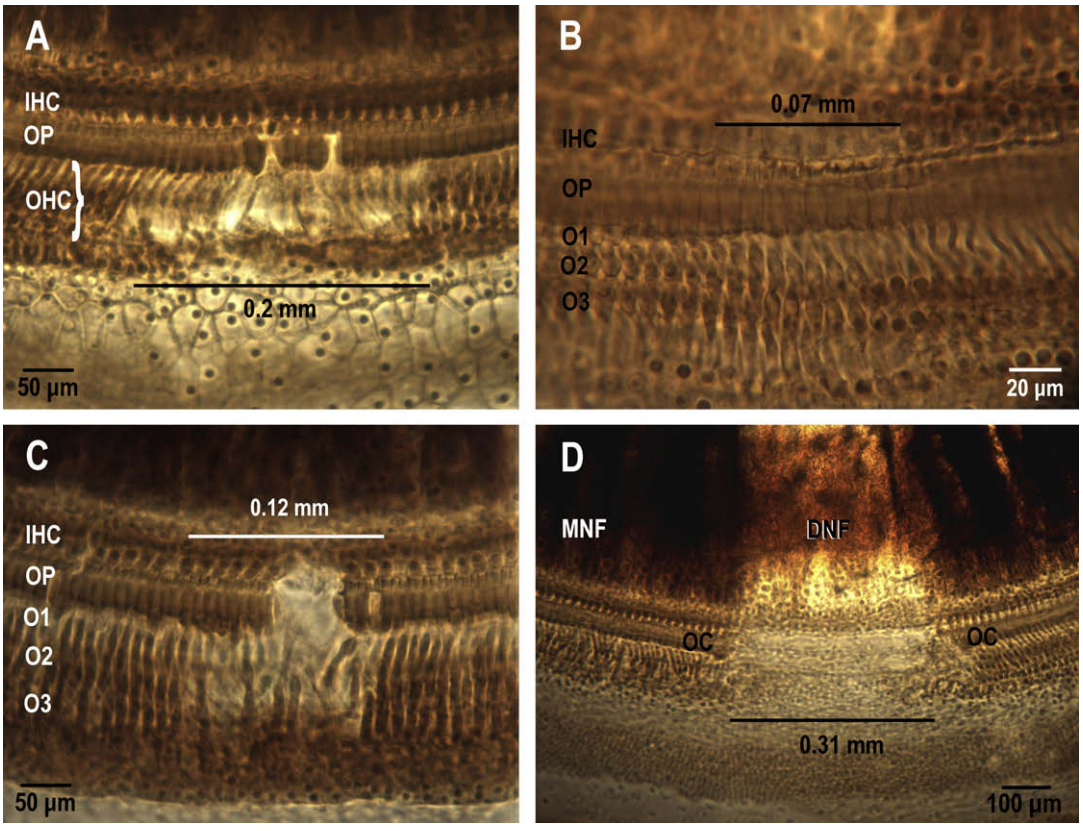

Fig. 1. Flat preparations showing appearance of different types of focal lesions and their size: (A) OHC; (B) pure IHC; (C) combined OHC and IHC; and (D) complete loss of the OC (OC-Wipeout). DNF – degenerated nerve fibers; IHC – inner hair cells; MNF – myelinated nerve fibers; O1, O2, and O3 – outer hair cells in rows 1, 2, and 3, respectively; OC – organ of Corti; OHC – outer hair cells; OP – heads of outer pillar cells.

Table 2
Number of cochleae and focal lesions by OBN, recovery group, and cluster number (Cl #) for OHC plus combined OHC–IHC (OHC+) and pure IHC lesions.

	# Cochleae	# Cochleae w/lesions (%)	# OCH+ lesions	# IHC lesions
Controls	140	55 (39)	56	130
4-kHz OBN				
Acute				
Cl 1	71	31 (44)	44	23
Cl 2	21	21 (100)	60	1
Cl 3	11	11 (100)	13	0
Chronic				
Cl 1	88	62 (70)	116	60
Cl 2	16	16 (100)	44	7
Cl 3	93	93 (100)	356	145
Interrupted				
Cl 1	46	23 (50)	22	39
Cl 2	1	1 (100)	9	1
Total	347	258	664	276
0.5-kHz OBN				
Acute				
Cl 1	58	18 (31)	22	10
Cl 2	3	3 (100)	14	10
Cl 3	7	7 (100)	9	0
Chronic				
Cl 1	102	84 (82)	276	154
Cl 2	38	35 (92)	254	26
Cl 3	18	18 (100)	23	1
Interrupted				
Cl 1	80	39 (49)	32	34
Cl 2	7	6 (86)	15	0
Total	313	210	645	235

340 animals that had been continuously exposed to either a 4-kHz (n = 190) or a 0.5-kHz OBN (n = 150). In addition, cochleae from 79 animals that had been exposed to these OBNs (28 and 51 animals, respectively) on interrupted schedules were included in the analysis (47 and 87 cochleae, respectively). The continuously exposed cochleae were grouped according to OBN and recovery time. The cochleae exposed on interrupted schedules were treated as separate groups by OBN. For each OBN and group, a cluster analysis using the K-means method (K = 3; Wilkinson, 1988) was performed for percent OHC loss in the basal half of the OC with the 4-kHz OBN and apical half of the OC with the 0.5-kHz OBN as a function of total energy in the exposure (Pa^2 s) as described in Harding and Bohne (2004a). This process analytically assigned each cochlea to one of three clusters based upon the percent OHC loss in its OC rather than the specific noise exposure to which the cochlea had been subjected. This approach reduced the inter-animal differences in hair-cell losses due to variations in susceptibility to noise (i.e., noise-sensitive, noise-susceptible, and noise-resistant animals). The expectation is that, with high-level, short-duration exposures, noise-resistant cochleae are in cluster 1, noise-susceptible cochleae in cluster 2, and noise-sensitive cochleae in cluster 3. With moderate-level, moderate-duration exposures, noise-resistant and noise-susceptible cochleae are in cluster 1 and noise-sensitive cochleae in cluster 2.

Focal lesion type was encoded as IHC (Fig. 1B) or OHC+, the latter including OHC lesions (Fig. 1A), combined OHC–IHC lesions (Fig. 1C), and OC-Wipeouts (Fig. 1D). These three lesion types were encoded together because it has been shown that it is very likely that they all began as predominantly OHC lesions (Harding and Bohne, 2007) and that OC-Wipeouts are extreme cases of combined lesions (see Section 3 for further justification). Focal-lesion location was quantified with respect to the basal edge, apical edge, and center of the lesion in percent distance from the OC apex. Focal-lesion size was measured in mm and converted to percent of OC length.

2.4. Data analysis

Lesion numbers and sizes (in mm) were statistically analyzed using SYSTAT 6.0 (Wilkinson, 1988) for the basal and apical halves of the OC. Significant differences were tested with the Kruskal–Wallis (K–W) test for lesion counts; an analysis of variance test for rank-ordered data using the Chi square statistic (Wilkinson, 1988). The K–W test was used to compensate for highly skewed count distributions. Standard analysis of variance (ANOVA) with factorial design was used for lesion sizes. The level for accepting significant differences was $p < 0.05$ in both cases. The data were also tallied for each OBN, recovery group, cluster, and lesion type in 2%-distance bins (i.e., 0.37 mm for an average OC length of 18.4 mm) with each lesion being weighted according to its size in percent of total length. The lesions were distributed proportionally across adjacent bins as needed to account for specific location and size. The results were expressed as percent of OBN-group-cluster total sample sizes (i.e., the number of OCs with and without lesions).

The original and subsequent studies which supplied the data for this retrospective analysis were conducted in accordance with the guidelines for animal research by the Acoustical Society of America and were reviewed and approved by Washington University's Animal Studies Committee (B.A. Bohne, PI).

3. Results

In general, cluster 1 contained cochleae subjected to moderate-level, moderate-duration noise exposures while clusters 2 and 3 mostly contained cochleae subjected to high-level, short-duration exposures. However, some high-level, short-duration exposed cochleae from noise-resistant animals were in cluster 1 (i.e., 54 of 191 {28%} cochleae for the 4-kHz OBN; 6 of 34 {18%} for the 0.5-kHz OBN) and a few moderate-level, moderate-duration exposed cochleae from noise-sensitive animals were in cluster 2 (i.e., 3 of 109 {3%} cochleae for the 4-kHz OBN; 36 of 191 {19%} for the 0.5-kHz OBN).

3.1. Numbers of focal lesions

The number of cochleae with lesions and the number of OHC+ and IHC lesions by OBN, recovery group, and cluster are shown in Table 2. In the controls, 39% contained focal lesions and 70% of these lesions involved IHCs only. The lesion type for 73% of the OHC+ lesions was OHC. The non-noise-exposed animals included some up to 12 y-old because the OC from more than half of the 12-y-old controls had no focal lesions. For all of the cochleae exposed to the 4-kHz OBN, 74% had focal lesions and 71% of the lesions were OHC+. The lesion type for 60% of these OHC+ lesions was OHC. For all of the cochleae exposed to the 0.5-kHz OBN, 67% had focal lesions and 73% of the lesions were OHC+. The lesion type for 53% of these OHC+ lesions was OHC. For both OBNs, all or nearly all cochleae in clusters 2 and 3 had focal lesions and they were predominantly OHC+ lesions.

The statistics for the mean number of focal lesions per cochlea with lesions by type, OBN, recovery group, and cluster are shown in Table 3 for the basal and apical halves of the OC. For many individual cases, the standard deviations were too large and/or the sample sizes were too small to find statistically significant differences between controls and noise-exposed cochleae, acute and chronic groups, the two OBNs, or basal and apical halves of the OC. Nonetheless, in the controls, there were about twice as many focal lesions (total) in the apical half of the OC compared to the

Table 3
Mean number of lesions, (s.d.), and *n* in cochleae with lesions by OBN, recovery group, and cluster (Cl #) in the basal and apical halves of the OC for OHC plus combined OHC–IHC (OHC+) and pure IHC lesions.

	Basal OHC+	Basal IHC	Apical OHC+	Apical IHC
Controls	1.5 (0.7), 16	1.7 (1.6), 25	3.6 (5.7), 9	3.2 (5.1), 27
4-kHz OBN				
Acute				
Cl 1	1.7 (0.9), 26	2.8 (2.0),[a] 8	0	1.0 (–), 1
Cl 2	2.8 (1.8),[a] 21	1.0 (–), 1	1.0 (0), 2	0
Cl 3	1.0 (0),[a] 11	0	1.0 (0), 2	0
Chronic				
Cl 1	1.9 (1.0), 56	3.6 (6.4), 16	1.0 (0), 8	1.0 (0), 3
Cl 2	2.3 (1.8), 16	1.7 (1.2), 3	1.6 (0.9), 5	2.0 (–), 1
Cl 3	1.1 (0.4),[a] 93	1.0 (0), 2	4.2 (3.6), 61	5.5 (4.9),[a] 26
Interrupted				
Cl 1	1.2 (0.4), 15	3.3 (5.9), 9	1.0 (0), 4	1.5 (0.5), 6
Cl 2	6.0 (–), 1	1.0 (–), 1	3.0 (–), 1	0
0.5-kHz OBN				
Acute				
Cl 1	1.6 (0.9), 8	1.6 (0.9), 5	1.1 (0.4), 8	1.0 (0), 2
Cl 2	1.5 (0.7), 2	10.0 (–), 1	3.7 (1.2), 3	0
Cl 3	1.0 (0), 7	0	1.0 (0), 2	0
Chronic				
Cl 1	2.3 (1.6),[a] 67	3.0 (3.7), 34	2.9 (3.0),[b] 42	3.5 (6.9), 15
Cl 2	2.9 (2.2),[a] 33	1.6 (1.4),[b] 13	4.8 (3.7),[a] 33	1.3 (0.5), 4
Cl 3	1.0 (0),[a] 17	1.0 (–), 1	1.5 (0.6), 4	0
Interrupted				
Cl 1	1.2 (0.4), 23	1.4 (0.7), 17	1.3 (0.5), 4	1.2 (0.4),[a] 9
Cl 2	1.0 (0), 4	0	1.8 (0.8), 6	0

[a] Significantly different from controls (K–W, $p < 0.05$; see text for specific values).
[b] Significantly different from acute (K–W, $p < 0.05$; see text for specific values).

Table 4
Mean lesion size (mm), (s.d.), and *n* in cochleae with lesions by OBN, recovery group, and cluster (Cl #) in the basal and apical halves of the OC for OHC plus combined OHC–IHC (OHC+) and pure IHC lesions.

	Basal OHC+	Basal IHC	Apical OHC+	Apical IHC
Controls	0.22 (0.26), 24	0.11 (0.21), 43	0.17 (0.31), 32	0.07 (0.05), 87
4-kHz OBN				
Acute				
Cl 1	0.24 (0.19), 44	0.09 (0.05), 22	0	0.05 (–), 1
Cl 2	0.90 (1.32),[a] 58	0.04 (–), 1	0.24 (0.11), 2	0
Cl 3	9.22 (3.32),[a] 11	0	2.88 (0.77),[a] 2	0
Chronic				
Cl 1	0.39 (0.33),[a,b] 108	0.12 (0.12), 57	0.25 (0.37), 8	0.05 (0.01), 3
Cl 2	2.41 (2.67),[a,b] 36	0.06 (0.01), 5	0.21 (0.16), 8	0.05 (0.01), 2
Cl 3	10.79 (4.09),[a] 102	0.35 (0.38), 2	0.41 (0.84),[b] 254	0.14 (0.24),[a] 143
Interrupted				
Cl 1	0.13 (0.09), 18	0.08 (0.05), 30	0.07 (0.04), 4	0.06 (0.06), 9
Cl 2	0.41 (0.60),[a] 6	0.06 (–), 1	3.16 (4.06), 3	0
0.5-kHz OBN				
Acute				
Cl 1	0.16 (0.12), 13	0.07 (0.03), 8	0.16 (0.15), 9	0.06 (0.00), 2
Cl 2	0.23 (0.11), 3	0.06 (0.02), 10	0.27 (0.32), 11	0
Cl 3	12.79 (1.44),[a] 7	0	1.67 (0.13),[a] 2	0
Chronic				
Cl 1	0.24 (0.26), 156	0.09 (0.09), 101	0.18 (0.25), 120	0.08 (0.06), 53
Cl 2	1.07 (2.54), 96	0.07 (0.06), 21	0.52 (0.96),[a] 158	0.24 (0.40),[a] 5
Cl 3	16.04 (1.53),[a,b] 17	0.17 (–), 1	2.20 (3.84),[a] 6	0
Interrupted				
Cl 1	0.47 (0.79), 27	0.09 (0.09), 23	0.11 (0.07), 5	0.06 (0.02), 11
Cl 2	0.84 (0.94),[a] 4	0	0.61 (0.93),[a] 11	0

[a] Significantly different from controls (ANOVA, $p < 0.05$; see text for specific values).
[b] Significantly different from acute (ANOVA, $p < 0.05$; see text for specific values).

basal half. Although not significantly different, the mean number of lesions per cochlea with lesions appeared to be larger in the apical half of the OC than in the basal half. The vast majority were pure IHC lesions. The existence of lesions in non-noise-exposed controls indicates that some of the lesions were age-related and those found in noise-exposed cochleae could have been pre-existing. However, with the exception of cellular debris in the hair-cell region which often persists for several days after cell death, there was no way to reliably distinguish between specific cases of pre-existing and noise-induced hair-cell death.

For both OBNs, the mean number of focal lesions per cochlea with lesions was similar to those in the controls. For the 4-kHz OBN, the exceptions were more OHC+ lesions in basal acute cluster 2 (K–W, $p < 0.02$), pure IHC lesions in basal acute cluster 1 ($p < 0.04$), and pure IHC lesions in apical chronic cluster 3 ($p < 0.002$). For the 0.5-kHz OBN, the exceptions were more OHC+ lesions in basal chronic clusters 1 and 2 ($p < 0.02$) and apical chronic cluster 2 ($p < 0.05$). There were fewer IHC lesions in apical interrupted cluster 1 ($p < 0.03$). In the basal half of the OC for both OBNs, the number of OHC+ lesions in cluster 3 was significantly less than in controls ($p < 0.03$). However, the sizes of many OHC+ lesions in cluster 2 and nearly all of the OHC+ lesions in cluster 3 were very large compared to those in controls and cluster 1 (see below). In addition, the OC apex to base, size-weighted distribution of OHC+ and IHC lesions for both OBNs was quite different than for the controls (see below). In general, there was not a substantial increase in the number of lesions post-exposure with the exception of apical OHC+ lesions for the 0.5-kHz OBN, chronic cluster 1 ($p < 0.03$).

3.2. Sizes of focal lesions

The statistics for the mean size of focal lesions (in mm) by type, OBN, recovery group, and cluster are shown in Table 4 for the basal and apical halves of the OC. As for the numbers of lesions, the standard deviations were too large and/or the sample sizes were too small in many individual cases to find statistically significant differences between controls and noise-exposed cochleae, acute and chronic exposures, the two OBNs, or the basal and apical halves of the OC. Nonetheless, in the controls, focal lesions were quite small and OHC+ lesions appeared to be about two times larger than IHC lesions.

For the 4-kHz OBN acute group, mean lesion size in cluster 1 was much like that in the controls for both halves of the OC. However, lesions in the apical half of the OC were rare. The size of OHC+ lesions for basal acute clusters 2 and 3 and chronic clusters 1–3 were larger than in controls (ANOVA, $p < 0.02$). The size of OHC+ lesions was so large in cluster 3, that they could not be considered focal. Although the sample size was small, a significant increase in lesion size compared to controls ($p < 0.001$) was found for OHC+ in acute cluster 3 in the apical half of the OC. In the interrupted group, mean lesion sizes were generally similar to those in the acute group and controls rather than those in the chronic group for both halves of the OC. A significant increase in mean lesion size post-exposure was found for basal OHC+ chronic clusters 1 and 2 ($p < 0.005$) and apical OHC+ acute cluster 3 ($p < 0.001$).

For the 0.5-kHz OBN, mean lesion sizes in both halves of the OC for the acute group were similar to those for the 4-kHz OBN, with those in cluster 1 being essentially the same as those in the controls. However, the mean size for both basal and apical OHC+ lesions for cluster 3 in the acute and chronic groups was significantly larger than in controls ($p < 0.003$). The mean size of apical OHC+ lesions in chronic cluster 2 ($p < 0.04$) and IHC lesions in chronic cluster 2 ($p < 0.001$) were also significantly larger than

in the controls. The mean size for OHC+ lesions in the interrupted group, cluster 2 was significantly larger than in controls for the basal and apical halves of the OC ($p < 0.008$ and $p < 0.024$, respectively). With the only exception noted above, the mean size of IHC lesions in both halves of the OC, and for all clusters, were similar to those in controls. The only significant increase in mean lesion size post-exposure was found for basal OHC+ lesions in chronic cluster 3 ($p < 0.001$).

Analysis of variance for lesion size with the 4-kHz OBN for the basal versus apical halves of the OC and lesion type (i.e., acute versus chronic for each cluster) showed no interaction for clusters 1 and 2 and a significant probability for lesion type (ANOVA, $p < 0.007$). For cluster 3, the independent and interactive probabilities were significant ($p < 0.0001$). Comparing these variables between the two OBNs, only lesion type had a significant probability ($p < 0.03$). For the 0.5-kHz OBN, only lesion type had a significant probability ($p < 0.0001$) in clusters 1 and 2. Cluster 3 could not be tested due to singularity in the covariance matrix. Comparing these variables between the two OBNs, both apical versus basal half of the OC and lesion type had significant probabilities ($p < 0.03$, $p < 0.006$, respectively) and the interaction was significant ($p < 0.03$). Comparing lesion size between acute versus chronic groups and cluster for each OBN showed that the independent and interactive probabilities were highly significant ($p < 0.0004$) for the 4-kHz OBN and only the independent probabilities were significant for the 0.5-kHz OBN ($p < 0.007$).

3.3. Distribution of focal lesions

The distribution of the size-weighted results in percent of total OC length are shown in Fig. 2 for the control cochleae and Figs. 3–5 for the noise-exposed cochleae. Note that the *y*-axis scale for the controls and cluster 1 noise-exposed cochleae is 5 times greater than that for clusters 2 and 3. In each graph, *m* = number of cochleae (with and without focal lesions) and *n* = number of focal lesions. The relation between percent distance from the apex and frequency place is based on the equation from Eldredge et al. (1981).

Focal lesions were found in non-noise-exposed cochleae. However, they were very small and were dominated by pure IHC lesions. In the controls, size-weighted OHC+ and pure IHC focal lesions were uniformly distributed in the OC with a slightly higher density at the basal and apical tips (Fig. 2). In the noise-exposed animals (Figs. 3–5), a few focal lesions could have been pre-existing, particularly the pure IHC focal lesions. However, with one exception (i.e., 0.5-kHz OBN, chronic, cluster 1), the distribution of focal lesions in the noise-exposed cochleae (see below) was quite different from that in controls.

Immediately after exposure to a 4-kHz OBN at a moderate level and for a moderate duration (Fig. 3; acute, cluster 1), a modest

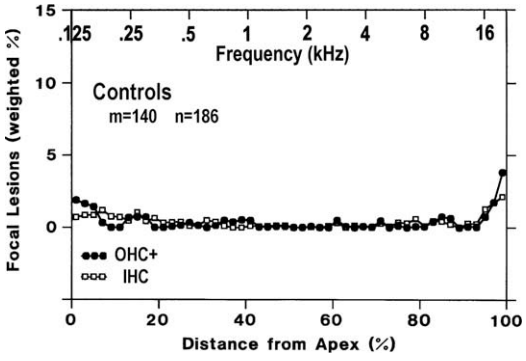

Fig. 2. Distribution for size-weighted percent of total OHC+ (closed circles) and IHC (open squares) focal lesions from non-noise-exposed controls. *m*, total number of cochleae; *n*, number of lesions.

number of OHC+ focal lesions were found primarily in and one-half octave above the frequency range of the OBN. Pure IHC focal lesions were less common. With recovery time (Fig. 3; chronic, cluster 1), many more OHC+ focal lesions were found with a substantial number located an octave or more above and below the frequency range of the OBN. The OHC+ focal lesions also increased in size. Pure IHC focal lesions increased in number and were also more widely distributed. The IHC focal lesions did not appear to increase in size post-exposure. Generally, with high-level, short-duration exposures, considerably more OHC+ focal lesions were found and they were more widely distributed (Fig. 3; acute, cluster 2). The size of lesions increased with recovery time (Fig. 3; chronic, cluster 2). In animals that were very sensitive to high-level, short-duration exposures, the lesions were so large (i.e., >4 mm) that they could not be considered focal. Acutely, there was nearly complete OHC loss, along with most IHCs, in the basal half of the OC (Fig. 3; acute, cluster 3). Pure IHC lesions were absent. It is likely that the OHC+ lesions started as several closely spaced focal lesions that grew in size and merged into a single large lesion. With recovery (Fig. 3; chronic, cluster 3), the damage extended further toward the apex. Some pure IHC focal lesions were found apical to the large combined OHC–IHC lesions.

Immediately after exposure to a 0.5-kHz OBN at a moderate level and for a moderate duration (Fig. 4; acute, cluster 1), OHC+ focal lesions were uncommon. However, some lesions occurred just below and about one-half octave above the frequency range of the OBN, as well as in the same basal region damaged by the 4-kHz OBN. Pure IHC focal lesions were rare. With recovery (Fig. 4; chronic, cluster 1), many more and larger OHC+ focal lesions were found in both OBN regions. A modest number of pure IHC focal lesions were found throughout the OC. Generally, with high-level, short-duration exposures, more and larger OHC+ focal lesions developed in the same pattern as seen acutely, particularly at a location apical to the OBN (Fig. 4; acute, cluster 2). The number and size of lesions increased with recovery time and substantial numbers of focal lesions developed well basal to the OBN, including in the 4-kHz OBN region (Fig. 4; chronic, cluster 2). A few pure IHC focal lesions also appeared in the 0.5- and 4-kHz regions. In animals that were very sensitive to high-level, short-duration exposures, OHC+ lesions were too large to be called focal. Acutely, there was nearly complete loss of OHCs and IHCs, in the basal two-thirds of the OC (Fig. 4; acute, cluster 3). With recovery time, the damage spread further toward the apex (Fig. 4; chronic, cluster 3). Pure IHC focal lesions were rare. For both OBNs, there was a bump apical to the leading edge of the massive OHC loss from high-level, short-duration exposures in the acute groups (Figs. 3 and 4, acute, cluster 3) which was also somewhat apparent in the 4-kHz chronic group (Fig. 3, chronic, cluster 3).

Fig. 5 shows the distributions of focal lesions that were found in cochleae that had been subjected to a 4- or 0.5-kHz OBN on interrupted schedules (i.e., moderate-level, moderate-duration). For the 4-kHz OBN, the distribution of focal lesions was similar to that of the continuous 4-kHz OBN (i.e., Fig. 3, chronic, cluster 1), but the magnitude was considerably reduced. For the interrupted 0.5-kHz OBN, focal lesions were virtually eliminated in the apical half of the OC. However, in the basal half, focal lesions occurred in nearly the same distribution as seen chronically for the continuous 4-kHz OBN exposure (i.e., Fig. 3; chronic, cluster 1), but at about half the magnitude. This distribution was very similar to that for the 4-kHz OBN acute group (i.e., Fig. 3; acute, cluster 1).

4. Discussion

Focal lesions were found in 39% of non-noise-exposed cochleae. Unlike those in the noise-exposed cochleae, these lesions were dominantly very small, pure IHC lesions. With the exception of

the basal and apical tips, focal lesions in the controls were uniformly distributed. It is possible that a few of the lesions found in the noise-exposed cochleae could have been pre-existing. Thus, any errors introduced in the analysis of the noise-induced-lesion data were small and constant from near the base to near the apex of the OC. In addition, the findings in the controls suggest that the same mechanisms for the formation of noise-induced focal lesions may be involved in age-related focal lesions. Although there is a high correlation between the magnitudes of total hair-cell loss in the right and left OCs in noise-exposed and aging chinchillas (Bohne et al., 1986), data from both cochleae were used in the present study because the numbers, sizes and locations of focal lesions in an animal's two cochleae are somewhat more variable.

From this analysis, it is clear that exposure level makes a difference in the development of focal lesions. With the 4-kHz OBN at a moderate level and moderate duration (i.e., cluster 1), 44% of the cochleae developed one or more focal lesions during the exposure. The proportion of cochleae with lesions increased to 70% post-exposure. With high-level, short-duration exposures (i.e., clusters 2 and 3), all cochleae had focal lesions regardless of recovery time. For the 0.5-kHz OBN, the results were similar; 31% of the cochleae during and 83% after a moderate-level, moderate-duration exposure and all or nearly all after a high-level, short-duration exposure. The chinchilla ear canal resonates over the entire 4-kHz OBN which adds about 16 dB SPL at the eardrum (Von Bismarck, 1967; Harding and Bohne, 2007). Thus, the total energy applied to the OC by a 4-kHz OBN at 80 dB SPL is comparable to that from a 0.5-kHz OBN at 95 dB SPL. The data shown in Figs. 3–5 can be loosely interpreted as the probability that a given OBN exposure will produce a focal lesion at a particular frequency place in the OC. These observations suggest that there are at least two mechanisms involved in the formation of noise-induced focal lesions. One mechanism is active with both moderate- and high-level exposures while a second is added with high-level exposures. The latter

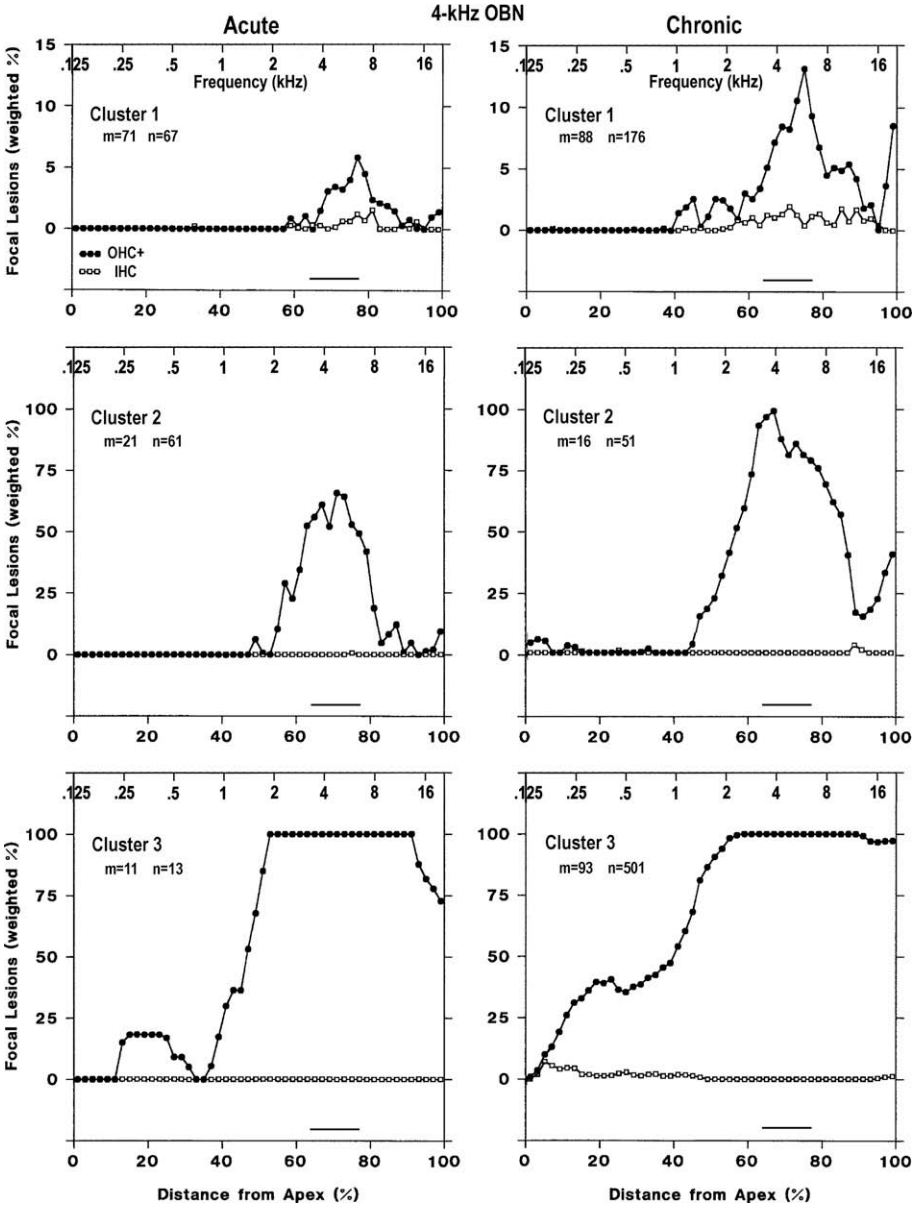

Fig. 3. Distribution for size-weighted percent of total OHC+ (closed circles) and IHC (open squares) focal lesions from continuous 4-kHz OBN by recovery group (acute, chronic) and cluster number. *m*, total number of cochleae; *n*, number of lesions; *x*-axis bar, OBN frequency range.

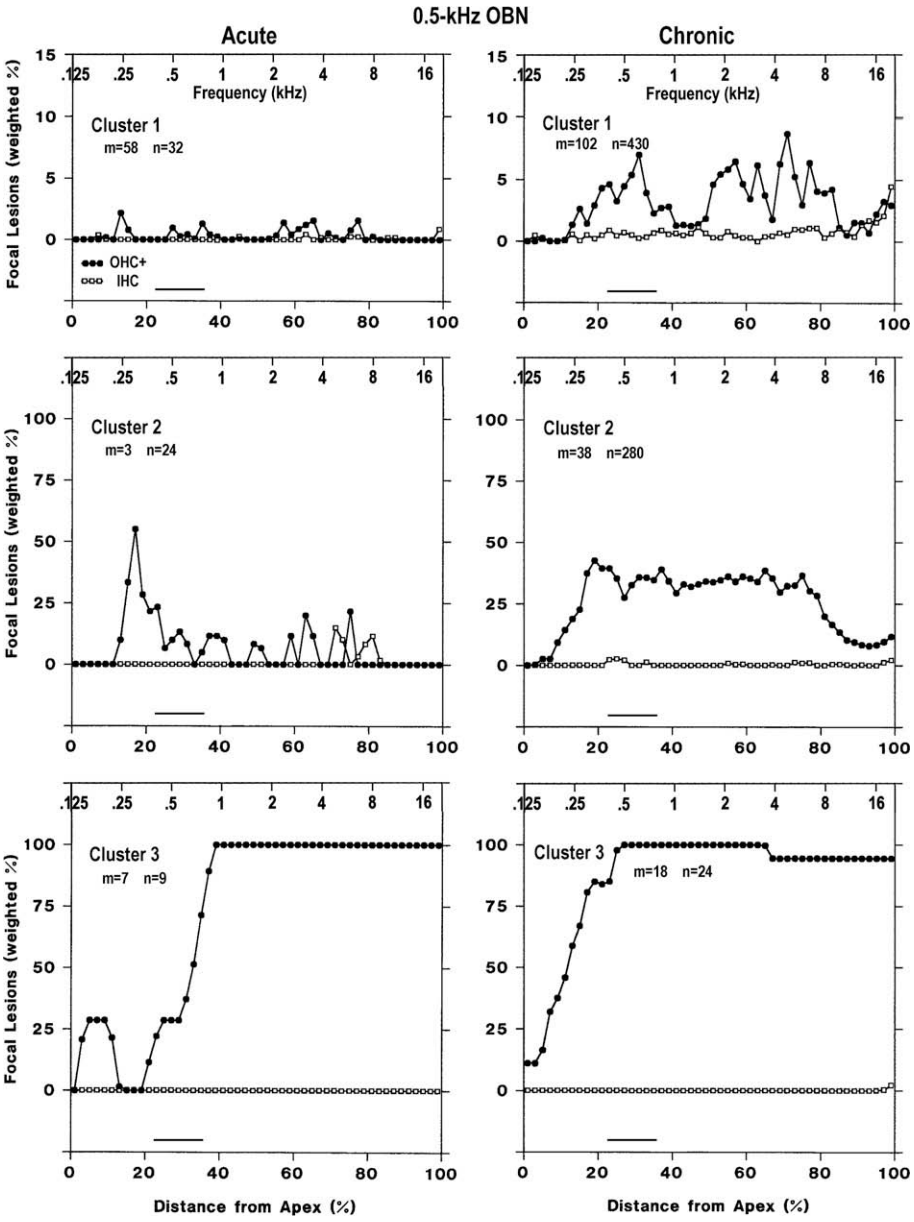

Fig. 4. Distribution for size-weighted percent of total OHC+ (closed circles) and IHC (open squares) focal lesions from continuous 0.5-kHz OBN by recovery group (acute, chronic) and cluster number. *m*, total number of cochleae; *n*, number of lesions; *x*-axis bar, OBN frequency range.

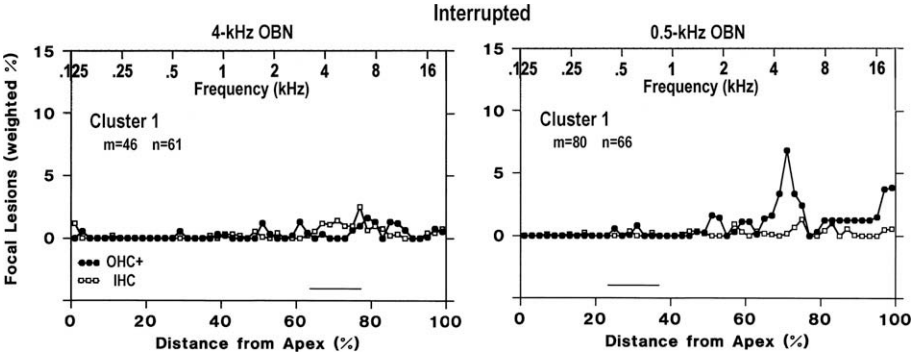

Fig. 5. Distribution for percent of total OHC+ (closed circles) and IHC (open squares) focal lesions from interrupted 4-kHz and 0.5-kHz OBN for cluster 1. *m*, total number of cochleae; *n*, number of lesions; *x*-axis bar, OBN frequency range.

mechanism generally produces much larger and more broadly distributed lesions. In our view, the massive damage from high-level, short-duration exposures (including high-level impulse noise) confounds examinations of noise-induced hair-cell death.

It has been reported that the generation of reactive oxygen species (ROS) during and post-exposure is the mechanism responsible for noise-induced OHC death (e.g., Quirk et al., 1992; Nuttall, 1999; Ohlemiller et al., 1999; Yamashita et al., 2004). However, the spotty nature of focal-lesion distribution in cochleae with multiple lesions (e.g., Bohne and Harding, 2000; Harding et al., 2002; Ahmad et al., 2003) suggests that ROS is not likely to cause widely spaced focal lesions to develop.

As reported previously for both OBNs (Harding and Bohne, 2007), it is surprising that so many focal lesions occurred well above and below the exposure band. Harding and Bohne (2007) examined the distribution of small focal lesions (i.e., <1.5 mm) where the sum of exposure duration and recovery time was less than 12 days. All of the lesions being small, they were not weighted by lesion size and they were predominantly in the acute groups, cluster 1. Their distributions were very similar to those reported here for the size-weighted acute groups. The well-known half octave (basal) shift for the location of hearing loss from an OBN (e.g., Cody and Johnstone, 1981) cannot account for these results. In addition, neither anomalous spikes in the noise energy outside of the band nor harmonics and distortion products can explain this phenomenon (Harding and Bohne, 2007). The results presented here suggest that basilar membrane/organ of Corti mechanics may be much more complicated than previously thought.

With the exception of the 0.5-kHz OBN at moderate level and duration (cluster 1), the number of focal lesions per cochlea did not significantly increase post-exposure. These results are contrary to those reported earlier (Harding and Bohne, 2004a). In the latter report, it was found that general OHC loss in the basal half of the OC occurred primarily post-exposure with a 4-kHz OBN and during the exposure in the apical half for a 0.5-kHz OBN. In the present study, we found that OHC+ lesions in the apical half of the OC increased in number post-exposure with a 0.5-kHz OBN at a moderate level and duration, although their size did not increase. This finding indicates that it is not sufficient to determine noise-induced hair-cell loss over a broad region of the OC. It is important to know whether that loss is scattered or focal.

The results from the interrupted exposures for both OBN are surprising. Rest has been shown to be protective for noise-induced hair-cell loss in general (Bohne et al., 1985, 1987). It is clear from the present results that rest periods during the exposure substantially reduce the development of focal lesions in the basal half of the OC from high-frequency noise (e.g., a moderate-level, 4-kHz OBN). Also, rest reduces the formation of focal lesions in the apical half from a low-frequency noise (e.g., a moderate-level, 0.5-kHz OBN). However, compared to continuous low-frequency noise (e.g., a moderate-level, 0.5-kHz OBN), rest only partially protects against the formation of focal lesions in the basal half of the OC. It has been well-documented that a 0.5-kHz OBN produces hair-cell loss in the high-frequency region of the OC (e.g., Bohne and Harding, 2000). The present observations further support the notion that the mechanisms for the development of focal lesions in particular, and hair-cell loss in general, differ in the basal and apical halves of the OC.

Most of the cochleae in this sample that were collected over the past decade had been functionally tested for ABR threshold shifts and DPOAE level shifts immediately post-exposure and over recovery time (e.g., Nordmann et al., 2000; Harding et al., 2002; Harding and Bohne, 2004b). The findings from these data indicate that even a small amount of focal IHC loss will produce a PTS (Nordmann et al., 2000). In the present analysis, OHC and combined OHC–IHC lesions were taken together to examine mechanisms (see below). However, there was small to substantial IHC loss in OHC+ lesions, although the basal-apical extent of the IHC loss was less than that of the OHC loss. These IHC losses produced PTSs (e.g., Harding et al., 2002; Ahmad et al., 2003). Focal lesions that were predominantly OHC lesions did not produce a PTS or a permanent DPOAE level shift (PLS) unless the size of the lesion was very large (i.e., >2.5 mm). Likewise, PLS was present only in OHC+ lesions of similarly large size (Harding et al., 2002). These observations indicate that consideration of focal lesions is important in understanding the mechanisms of noise-induced hearing loss.

It is likely that predominantly OHC and combined focal lesions begin as a concentrated loss of OHCs. This initial loss leaves holes in the reticular lamina which allow endolymph to intermix with perilymph in the fluid spaces of the OC. The increased potassium concentration in Cortilymph induces oncosis in the adjacent OHCs, leading to further OHC death. The lesion can spread basally and apically to include more OHCs, and medially to involve IHCs, supporting cells, and nerve fibers, thus forming a combined OHC–IHC lesion. An OHC or OHC+ lesion stops growing when the repair processes have healed the damaged OC and reestablished the boundary between endolymph and the fluid spaces of the OC (Bohne and Rabbitt, 1983; Ahmad et al., 2003). The questions to be addressed in future research are: What is it about excessive noise exposure that initially kills OHCs in a focal pattern; What kills the IHCs in pure IHC focal lesions; and What causes continued formation of focal lesions post-exposure?

Noise-induced death pathways in hair cells are a controversial issue. Some investigators, using propidium iodide, TUNEL, and fluorescence methods, have presented data indicating that excessive noise leads to OHC death by apoptosis (e.g., Nicotera et al., 2003; Hu et al., 2006). Although these studies employed some high-level, 4-kHz OBN exposures, much of these results were based upon high-level impulse noise. Using plastic-embedded specimens and classic morphological criteria rather than wet dissections and immunofluoresence staining, we have found few apoptotic OHCs in noise damaged cochleae (Bohne et al., 2007; Lee et al., 2008). With the same animal model and a comparable OBN as used by Hu and colleagues, we find that OHC death by apoptosis is extremely rare, even with high-level exposures. Also, we do not find OHC death by apoptosis with moderate-level OBN. We argue that apoptosis, having a time course of hours between initiation and completion (i.e., Kerr et al., 1972; Majno and Joris, 1995; Cummings et al., 1997), cannot explain OHC loss that continues several weeks post-exposure. Rather, we have found evidence for initial noise-induced OHC death by a non-apoptotic, non-oncotic death pathway. Determination of the mechanism for this pathway will require further investigation. Thus far, we have found, using transmission electron microscopy, breaks in the apical membrane of OHCs (Schmitt et al., 2004). These breaks (i.e., adjacent to the endolymphatic space) would allow a constant current through OHCs; a current flow driven by the endolymphatic potential. Such a condition (unique to the cochlea) would be expected to quickly lead to metabolic exhaustion and ultimate OHC death. As for pure IHC focal lesions, the mechanism of IHC death is probably different from that for OHCs but, this mechanism remains a mystery.

5. Conclusions

1. With a high-level 4-kHz OBN, focal lesions cover the entire basal half of the OC. With a moderate-level 4-kHz OBN, focal lesions occur in the basal half of the OC and well below and above the OBN.
2. With a high-level 0.5-kHz OBN, focal lesions cover the basal two thirds of the OC. With a moderate-level 0.5-kHz OBN, focal lesions occur throughout the OC, below and well above the OBN.

3. Focal lesions begin to appear at relatively low exposure levels (i.e., 75–80 dB SPL) for both OBNs. Above a certain level (i.e., 92–95 dB SPL), all cochleae have focal lesions.
4. Noise-induced, pure IHC focal lesions are much less common and much smaller than OHC lesions.
5. Although OHC and combined OHC–IHC focal lesions can develop during moderate-duration exposures, most of them occur after the exposure is over.
6. With high-level exposures, the very large lesions begin as small focal lesions that enlarge and merge.
7. The mechanism for generation of focal lesions appears to be different for moderate- versus high-level exposures, for the basal versus the apical half of the OC, and for OHC and combined OHC–IHC versus pure IHC lesions.

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