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Noise-induced hair-cell loss and total exposure energy: Analysis of a large data set[a)]

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The relation between total noise-exposure energy, recovery time, or rest during the exposure and amount of hair-cell loss was examined in 416 chinchillas. The exposures were octave bands of noise (OBN) with a center frequency of either 4 kHz at 47–108 dB sound pressure level (SPL) for 0.5 h to 36 d, or 0.5 kHz at 65–128 dB SPL for 3.5 h to 432 d. Recovery times varied from 0 to 365 d. With both OBNs, some animals were exposed on interrupted schedules. Hair-cell loss as a function of age in nonexposed animals ($N=117$) was used to correct for sensory-cell loss due to aging. For both OBNs, the ears ($N=607$) were separated into three subsets to characterize the primary hair-cell loss from noise and the secondary post-exposure loss and to determine if rest during the exposure decreased loss. Cluster and regression analyses were performed on data from the basal and apical halves of the cochlea to determine the specific rates for these three factors. It was found that: (1) when the OBN was above a critical level, there was no relation between total energy and hair-cell loss; (2) below a critical level, there were highly significant log–linear relations between total energy and hair-cell loss, but not at rates predicted by the equal-energy hypothesis; (3) rest periods during either OBN exposure reduced hair-cell loss; more so for the 4 kHz OBN than the 0.5 kHz OBN; (4) except for the highest exposure levels, the majority of outer hair cell loss from the 4 kHz OBN occurred after the exposure had terminated, while that from the 0.5 kHz OBN occurred during the exposure; and (5) a majority of the inner hair cell loss from both OBNs occurred post-exposure.
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I. INTRODUCTION

The equal-energy hypothesis is an often accepted relation between noise exposure and noise-induced hearing loss (NIHL). The notion is that the same magnitude of NIHL is produced by a variety of noise exposures, each of which has equivalent total energy. That is, the frequency content, level, and duration of the noise exposure are unimportant as long as the total energy is the same. Commonly, a 3 dB tradeoff rule is applied to estimate this relation (e.g., Eldredge and Covell, 1958). To limit damage from any given noise frequency spectrum, the duration must be halved when the level is increased by 3 dB.

The equal-energy hypothesis can also be stated with respect to noise-induced hair-cell loss. The assumption here is that permanent threshold shift (PTS) is highly correlated with hair-cell loss. The expectation is that different exposures with equal total energy produce the same amount of hair-cell loss. However, a doubling of total energy does not necessarily produce a doubling of hair-cell loss. The outer hair cells (OHC) are the first sensory cells to show signs of noise-induced damage. As the exposure continues, the OHC loss can become substantial. Even so, the correlation of OHC loss with PTS is weak (e.g., Clark and Bohne, 1978). Exposure to noise also results in inner hair cell (IHC) loss, but it is generally secondary to OHC loss and its magnitude is considerably smaller. However, IHC loss correlates with PTS very well, particularly in cases of focal IHC loss (e.g., Nordmann *et al.*, 2000) and large losses (e.g., Harding *et al.*, 2002).

A number of studies have been conducted to test the equal-energy hypothesis, but the results have often been contradictory. Findings appeared to depend upon the animal model, noise-exposure, and functional testing paradigms as well as the methods for histopathological assessment. In addition, the results have been confounded by several other issues. In some cases, the animals were terminated immediately post-exposure when there was a substantial temporary threshold shift (TTS). It has recently been indicated that the mechanism leading to TTS is completely different from that for PTS (Nordmann *et al.*, 2000). The correlation of TTS with PTS or with total exposure energy is poor (e.g., Ward, 1973). Some studies used intense, short duration noise exposures which may have produced mechanical damage or acoustic trauma. Other studies involved moderate level exposures for longer durations which produced hair-cell loss by mechanisms other than mechanical damage. Ward *et al.* (1981) observed that the equal-energy hypothesis is not applicable in cases of acoustic trauma and introduced the ''critical level'' hypothesis. That is, if the intensity of the exposure is above a critical level, the equal-energy hypothesis does not apply.

To test the equal-energy hypothesis, some studies used relatively narrow ranges of exposure level and duration. Studies have employed several different exposure paradigms ranging from pure tones (e.g., Eldredge and Covell, 1958; Goulios and Robertson, 1983), narrow-band impact noise

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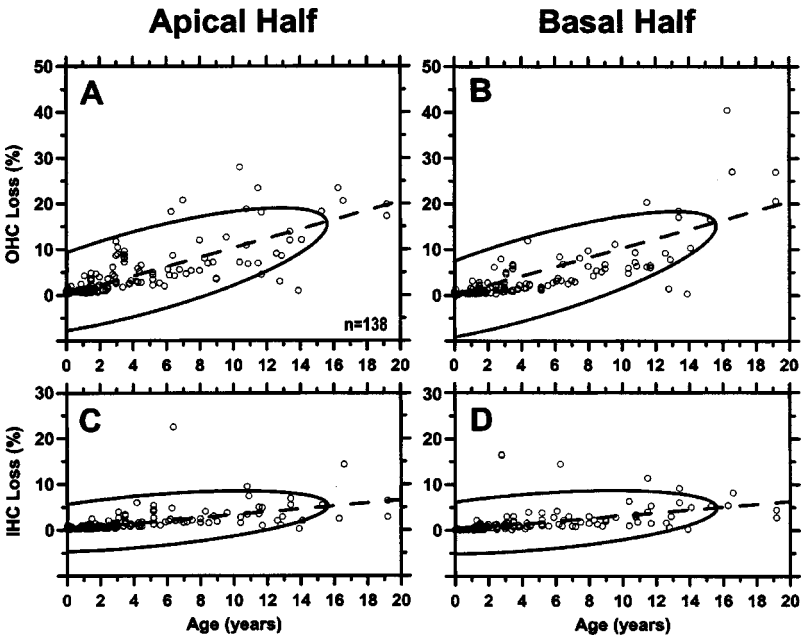

FIG. 1. Hair-cell loss due to aging for the OHC [(a) and (b)], and IHC [(c) and (d)] within the apical half [(a) and (c)] and basal half [(b) and (d)] of the OC. Regressions (q)—dashed lines; Gaussian bivariate ellipses at 0.95 probability level. Data from 117 chinchillas.

(e.g., Hamernik and Ahroon, 1998), octave bands of noise (OBN, e.g., Ahroon *et al.*, 1993; as well as impact noise), broad-band impact noise (e.g., Henderson *et al.*, 1982; 1991), and broad-band continuous noise (e.g., Salt *et al.*, 1981; as well as impact noise). Many previous studies were based upon a relatively small sample that included a large variance associated with the animals' susceptibility to noise damage. The possibility that noise affects the apical and basal halves of the organ of Corti (OC) differently was not considered. Previous studies did not distinguish between primary hair-cell loss that occurred during the exposure and secondary loss (e.g., Bohne and Harding, 2000) that continued for days post-exposure. Finally, age-related loss of hair cells was not taken into accounted.

The present study was conducted retrospectively using a large data set from the ears of chinchillas exposed to a 4 or a 0.5 kHz OBN with a wide range of levels and durations. The relation between exposure energy and noise-induced hair-cell loss was examined. The data set was large enough to determine the relevance of many of the above-noted issues and to determine the specific rates at which hair-cell loss occurred relative to total noise-exposure energy, rest during the exposure, and post-exposure recovery time.

II. DATA COLLECTION METHODS

From our collection of plastic-embedded chinchilla cochleae, the data from all non-noise-exposed control ears for animals aged from 0.2 to 19.2 yr and the data from 1 to 3 yr-old animals bilaterally exposed to noise were entered into a data set. The noise-exposed animals were subjected to an OBN with a center frequency of either 4 or 0.5 kHz in a reverberant booth on a continuous or interrupted schedule. Either immediately after the exposure or after a post-exposure recovery time, the cochleae were fixed with a buffered solution of osmium tetroxide, dehydrated, and embedded in plastic. After polymerization of the plastic, the cochleae were dissected into flat preparations from which missing hair cells were counted from apex to base and other pathology was quantified. These data were analyzed and cytocochleograms prepared relative to the percent location from the OC apex. See Bohne (1976), Clark *et al.* (1987), and Bohne *et al.* (1985; 1987; 1990) for details of noise exposures, histological processing, and quantification of hair-cell loss. The studies described here were conducted in accordance with the *ASA: Guiding principles for research involving humans or animal subjects* and were reviewed and approved by Washington University's Animal Studies Committee (Protocols #97100 and #20000131; B. A. Bohne, PI)

The information in the data set included: ear number; age (yr); OC length (mm); sound pressure level of the exposure (dB SPL); duration of exposure (total h); rest time during the exposure (total h; if any); recovery time post-exposure (d); percent IHC and OHC loss in the apical and basal halves of the OC; and an encoding of OC wipeouts, focal lesions, and nerve fiber loss in the apical and basal halves. OC wipeouts are regions of complete sensory and supporting cell loss (Bohne and Clark, 1982) and are always accompanied by nerve fiber loss that appears to be secondary to the loss of IHCs. Focal lesions are regions with at least 50% loss of OHCs, IHCs or both cell types over a distance of at least 0.03 mm (Bohne *et al.*, 1987). With substantial focal IHC loss, there is also nerve fiber loss (e.g., Bohne and Harding, 2000).

III. ANALYTICAL METHODS AND RESULTS

A. Hair-cell loss due to aging

Data from 138 non-noise-exposed ears from 117 animals, ranging in age from 0.2 to 19.2 yr, were used to determine the rate of hair-cell loss with age. Chinchillas have a life span of more than 15 yr. Figure 1 shows this relation for OHC (top) and IHC (bottom) loss in the apical [(a) and (c)] and basal [(b) and (d)] halves of the OC. Linear regressions by age were performed to determine the rates at which hair-cell loss (HCL) occurred:

$$\%\mathrm{HCL}_{\mathrm{AGE},h} = q_h x \text{ age}, \quad (1)$$

where HCL=OHC or IHC; h=OC region (basal or apical half); q=aging rate; age in years.

These rates per year were 1.010% for OHC loss and 0.346 for IHC loss in the apical half and 0.918 for OHC loss and 0.324 for IHC loss in the basal half of the OC (Pearson $r=0.862$, 0.694, 0.844, and 0.636, respectively). The apical and basal OHC losses were significantly different at the $p<0.0005$ level while the apical and basal IHC losses were not significantly different. The resulting relations were then subtracted, by OC region and hair-cell type, for the HCL data from the noise-exposed animals using Eq. (1) to remove age-related loss of hair cells. Negative values produced by this adjustment were retained to preserve the intrinsic variance. The data were plotted along with a Gaussian bivariate ellipse (derived from the eigen values of the co-variance matrix) at a probability level of 0.95. Note that the major axes of these ellipses do not correspond precisely with the regression lines. If not specified hereafter, base or basal and apex or apical refer to the basal half and the apical half, respectively. All analytical procedures were performed using SYSTAT (ver. 5.03, SYSTAT, INC, Evanston, IL). The level of statistical significance used was $p=0.05$.

B. Subsets of noise-exposed ears

The data from the noise-exposed animals were separated into subsets based upon the frequency band of the free-field exposure, the duration of exposure, the total amount of rest during an interrupted exposure, and the length of post-exposure recovery time. None of these animals were exposed at high levels for long durations. Rather, higher exposure levels were presented for shorter durations and lower levels for longer durations. For each OBN, the ears were divided into three subsets (Acute, Chronic, and Interrupted).

The Acute subset included ears exposed continuously for durations less than or equal to 9 d having a recovery time of 0 d. This subset represented the primary effects of the noise exposure (i.e., those which occurred during the exposure), minimizing overlap with secondary effects which developed with longer exposures and/or recovery times greater than 0 d. Possible secondary effects that would lead to continued degeneration in the OC include intermixing of cochlear fluids through a damaged reticular lamina (e.g., Ahmad *et al.*, 2003). With lower level, longer duration exposures, substantial secondary effects generally appear from 7 to 9 d after exposure onset.

The Chronic subset included ears exposed continuously for longer than 9 d (with any recovery time) and those ears with recovery times longer than 0 d. This subset represented the primary effects of the noise plus the secondary effects that either overlapped with the latter part of the exposure or occurred post-exposure.

The Interrupted subset included discontinuous exposures (6 h per d, 6 h per 2 d, or 6 h per week) and all recovery times. All ears in the Interrupted subset were exposed for 9 or more d. The primary and secondary effects of the exposure, rest, and recovery were expected to overlap. This subset represented the primary effects of the noise, minus the effects of rest, plus the secondary effects of recovery times greater than 0 d.

C. Noise exposures

The ears in each subset were exposed as shown in Tables I(a) and (b). Different symbols were assigned to each exposure level (or level range). Also listed are the exposure duration and post-exposure recovery time ranges and the number of ears and animals for each subset and level. The exposure levels for the 4 kHz OBN were generally lower than for the 0.5 kHz OBN because the chinchilla ear canal resonates at about 4 kHz, adding approximately 15 dB SPL at the eardrum and across the entire 4 kHz OBN (von Bismarck, 1967). For each OBN, the distribution of animals exposed at the different level and duration combinations was similar for the Acute and Chronic subsets.

For each ear, the total energy (E) to which it was exposed was calculated as follows: the exposure level was converted to pressure in pascals (Pa); the pressure was squared and multiplied by the exposure duration (excluding rest) in seconds resulting in the unit pascal squared seconds ($\mathrm{Pa}^2\,\mathrm{s}$). This unit is a common way to express exposure energy (e.g., Annex F in ANSI Standard S3.44, 1996). For example, the total energy in a 90 dB SPL, 8 h exposure is 11 520 $\mathrm{Pa}^2\,\mathrm{s}$. The log to the base 2 of the energy value was used as the independent variable [e.g., 13.5 for the above 11 520 (or $2^{13.5}$) $\mathrm{Pa}^2\,\mathrm{s}$ example]. The data were plotted on a linear X axis as powers of 2 to represent progressive doubling of total exposure energy. An example is shown in Fig. 2(a) for the noise-induced OHC loss in the basal half of the OC in the 4 kHz OBN Acute subset. For the 4 kHz OBN, exposure level was not corrected for ear-canal resonance so that the data could be plotted relative to total energy in the free field. A 15 dB SPL correction for resonance would require a multiplicative factor of $2^5\,\mathrm{Pa}^2$, which would increase the value of $\log_2(E)$ by five units.

The data in Fig. 2(a) suggest that the results could be fitted with a logistic model. However, such a model would assume that hair-cell loss has a single mechanism regardless of total energy. Because there is evidence that hair-cell loss from noise arises from more than one mechanism (e.g., Bohne and Harding, 2000), a different approach was used.

D. Cluster analysis

Inspection of a raw scatter plot (not shown) of the data for basal OHC loss from the 4 kHz OBN Acute subset clearly showed that there were three distinct groups. However, with the 108 dB SPL exposures, ears fell within each of these groups. Therefore, rather than assigning ears to a particular group based on exposure level, a cluster analysis was performed upon each subset using the K-means method (Hartigan, 1979) to separate the responses to noise, rest, and recovery into three clusters. With this method, the number of clusters must be specified beforehand. For animals having data from both ears where the two ears were initially assigned to different clusters, the ear with the lower cluster assignment was reassigned to the higher cluster. This was

TABLE I. Subsets by exposure band and exposure-level ranges, (a) 4 kHz OBN, (b) 0.5 kHz OBN.[a]

		Acute subset		Chronic subset		Interrupted subset	
	Exposure levels symbol	Exposure recovery	*N*: Ears (animals)	Exposure recovery	*N*: Ears (animals)	Exposure recovery (d)	*N*: Ears (animals)
(a)	47–72 dB SPL Pentagon	2–9 d 0 d	15 (8)	2–9 d 30 d	7 (5)	…	…
	80 dB SPL Diamond	2–9 d 0 d	10 (5)	1–9 d 6–30 d	12 (10)	36–252 0 or 30	24 (12)
	85–86 dB SPL Square	1–9 d 0 d	20 (13)	1–36 d 4–30 d	29 (22)	9–72 0–365	20 (16)
	108 dB SPL Circle	0.5–24 h 0 d	45 (33)	1–24 h 1–365 d	137 (91)	9[b] 365	2 (2)
	Total: Ears (animals)		90 (59)		185 (128)		46 (30)
(b)	65–85 dB SPL Pentagon and diamond	2–9 d 0 d	18 (9)	2–9 d 30 d	11 (8)	…	…
	95 dB SPL Square	2–9 d 0 d	27 (22)	2–433 d 7–913 d	103 (74)	36–252 0–105	79 (55)
	108 dB SPL Circle	2–9 d 0 d	4 (2)	9 d 30 d	2 (1)	9 or 36[c] 0 or 30	8 (4)
	120–128 dB SPL Triangle	3.5–14 h 0 d	7 (4)	3.5–13 h 30–730 d	27 (20)	…	…
	Total: Ears (animals)		56 (37)		143 (103)		87 (59)

[a]Grand total of 607 noise-exposed ears from 416 animals.
[b]At 92 dB SPL.
[c]At 101 dB SPL.

done so that susceptibility to noise was relative to individual animals rather than ears. After reassignment, ears ($N=15$ out of 622) that were more than four standard deviations away from the center of the cluster were treated as outliers and discarded. These latter instances showed clear evidence that the animal's two ears did not get the same exposure. It is likely that this problem arose when there was an undetected temporary or permanent conductive hearing loss in one ear but not the other.

The clusters were plotted with a Gaussian bivariate ellipse at a 0.95 probability level to enclose the majority of points within the cluster and indicate the degree of cluster overlap. Animals with two ears in the subset contributed more weight than animals with just one. However, it has been shown that there is a high correlation of hair-cell loss between bilaterally exposed chinchilla ears (Bohne *et al.*, 1986).

Figure 2(a) shows that there is no relation between OHC loss and total exposure energy for the 108 dB SPL exposure level (circles). These data form clusters 2 (blue) and 3 (red) with cluster 3 near the upper limit imposed by the ceiling effect. Cluster 3 appears to be correlated with total energy, but a regression on these data implies that a 1 Pa^2 s exposure would produce an unrealistic 70% OHC loss. Therefore, 108 dB SPL was considered to be above critical level. With exposures below 108 dB SPL, there was a clear relation between OHC loss and total energy in cluster 1 (green), which included a few noise-resistant animals exposed at 108 dB SPL. The occurrence of 108 dB SPL ears in cluster 1 required broadening the definition of critical level to include an additional mechanism for hair-cell loss; one which did not involve mechanical damage.

E. Model of noise-induced hair-cell loss

In cluster 1, the primary effect of the noise exposure on hair-cell loss was considered to be related to the total energy of the exposure. For 4 kHz OBN exposures, the most affected hair cells were the OHCs in the basal half of the OC. For 0.5 kHz OBN exposures, the most affected were the OHCs in the apical half (e.g., Bohne and Harding, 2000). The slope of the relation between hair-cell loss and total energy should be decreased by the amount of rest (R) during the exposure and increased by the amount of loss which occurred during post-exposure recovery time (P). However, there was a critical exposure level above which the relation did not apply. The following model was formed to accommodate these observations:

$$\%\mathrm{HCL}_h=[a_h \log_2(E)+k_h]+b_h \log_2(P)+c_h \log_2(R), \quad (2)$$

where HCL=IHC or OHC loss in the OC depending upon the OBN of the exposure, with the values of a, k, b, and c being determined by log–linear regression of HCL with the

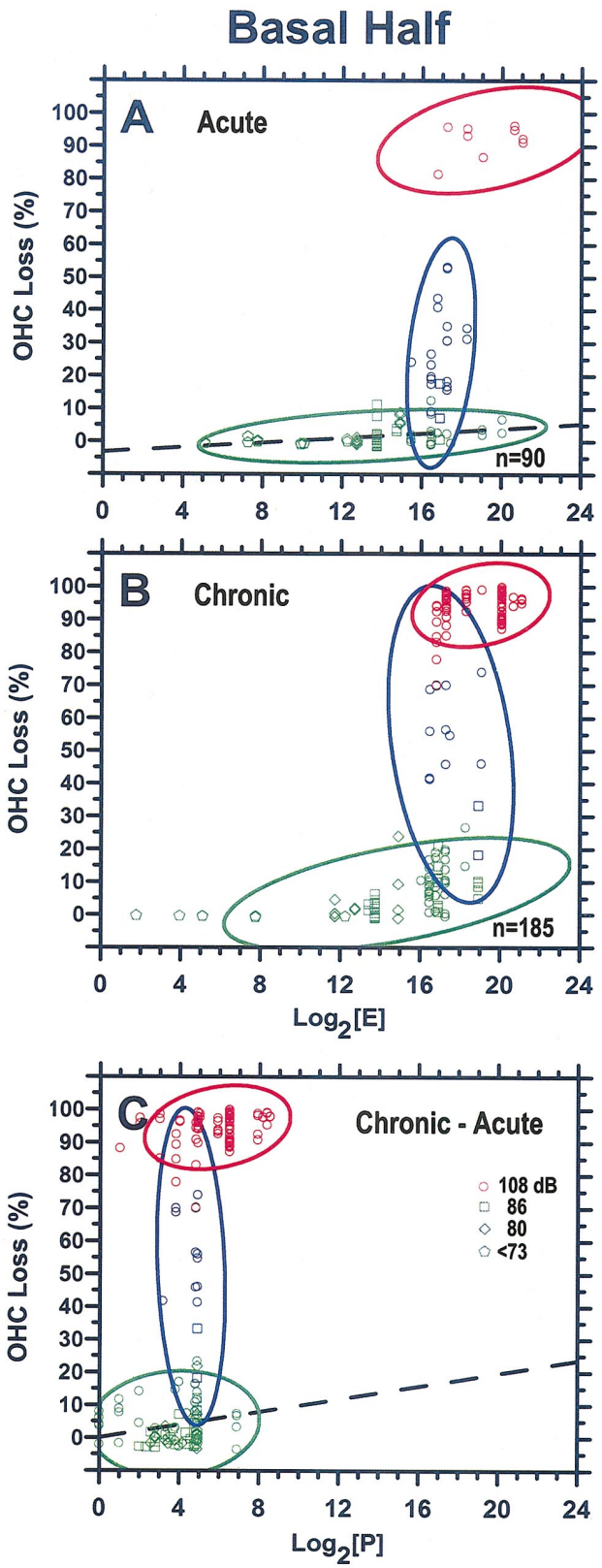

FIG. 2. 4 kHz OBN: (a) Acute subset: Cluster 1 (green) shows a strong relation (regression A) with total energy [E]. The exposures for clusters 2 (blue) and 3 (red) were above critical level and show no relation to E. (b) Chronic subset: (c) Subtraction of regression A from B for cluster 1 shows effect of post-exposure recovery time [P]. Symbol legend in (c) specifies exposure levels [see Table I(a)] and applies to (a)–(c). Gaussian bivariate ellipses at 0.95 probability level.

$\log_2$ of the independent variable from the data in cluster 1; h = OC region (basal or apical half).

The values of coefficient a and constant k were determined by regression [e.g., Fig. 2(a)] on the data for each of the 4 and 0.5 kHz OBN Acute subsets because E was known and both R and P were zero. Having determined a and k, the value for coefficient b was determined by regression [e.g., Fig. 2(c)] on the data for the Chronic subsets [Fig. 2(b)], cluster 1, after subtracting the Acute effect [Fig. 2(a)] using Eq. (2), with coefficients b and c equal to zero. Having determined the values for a, k, and b, the value of coefficient c was determined by regression (not shown) on the data in cluster 1 for the Interrupted subsets after subtracting the Acute and Chronic effects with Eq. (2). In the subtractions, any resulting negative values were retained to preserve the variance.

The data in Figs. 3–8 were plotted using the coefficients and independent variables on the right-hand side of Eq. (2) (without constant k) to determine the value of the independent (X axis) value. This maneuver was done to accommodate all three ongoing processes on the same scale simultaneously. For the Acute subsets, the value of coefficient a was as determined and coefficients b and c were zero. For the Chronic subsets, the values of coefficients a and b were as determined and coefficient c was zero. The P term added to the E term pushed the HCL data to the right relative to E by incorporating the duration of recovery time; moving the data slightly for shorter recovery times, more for longer recovery times. This procedure adjusted for the secondary HCL which occurred post-exposure. The relative contributions of the E and P terms can be appreciated by comparing Fig. 2(b) with Fig. 2(c). For the Interrupted subsets, the values of coefficients a, b, and c were as determined. In addition to the P term as above, the added term R pushed the HCL data to the left (because coefficient c should be negative) relative to E and P by incorporating the duration of rest during the exposure. The displacement was slight for less rest and more so for more rest. Note that plotted this way, the slopes of the major axes of the ellipses in Figs. 3–8 were changed.

F. The 4 kHz OBN

The results for the 4 kHz OBN Acute, Chronic, and Interrupted subsets are shown in Figs. 3, 4, and 5, respectively, and in Table II. The symbols in the figures represent exposure level as listed in Table I(a). Table II lists the number of ears and animals for each cluster of each subset along with the means and standard deviations for the independent variable [from the right-hand side of Eq. (2)], and the percent HCL for the basal OHC and IHC and apical OHC and IHC. Independent-, dependent-variable pairs of means are the locations of the centers for the ellipses enclosing each cluster.

Figure 3(a) shows the OHC loss in the Acute subset on an Eq. (2) scale for the basal half of the OC after cluster reassignment and outlier deletion. There were three distinct clusters as in Fig. 2(a). Cluster 1 showed a clear relation with total exposure energy. Coefficient a in Eq. (2) was 0.345 and constant k was -3.046 (Pearson $r = 0.366$). The effect of the scaling produced by coefficient a can be appreciated by com-

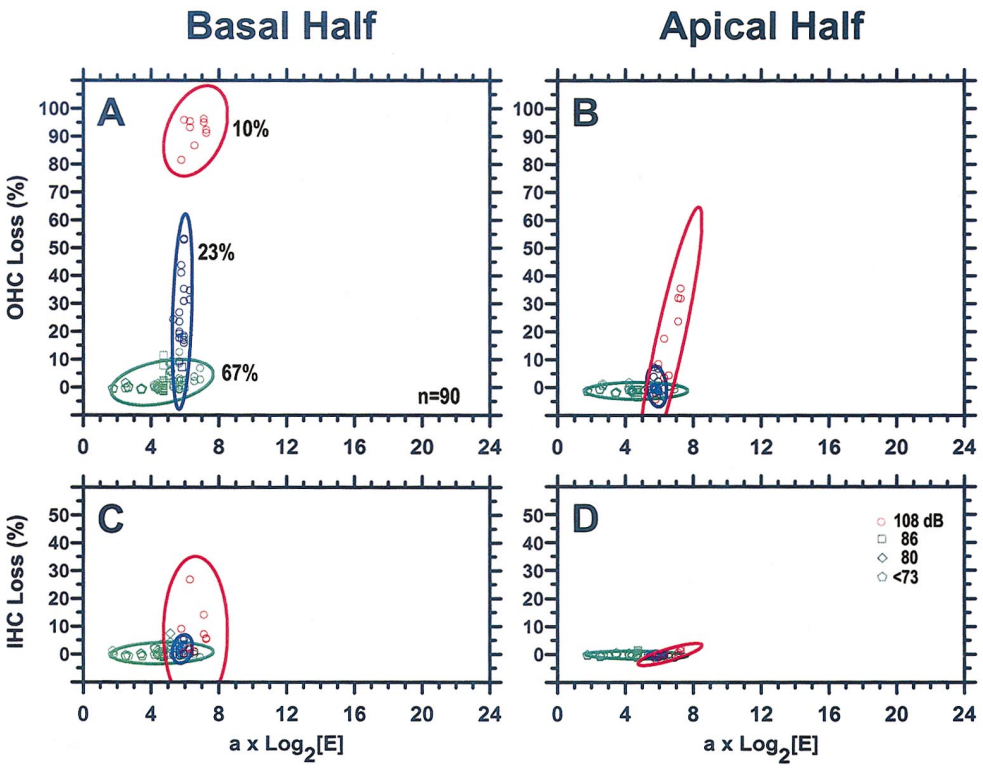

FIG. 3. 4 kHz OBN, Acute: (a) OHC—base: Below 108 dB SPL, there is a strong relation with total energy (green); 108 dB SPL (circles) is above critical level and shows no relation. (b) OHC—apex: Little OHC loss except that which spread up from base at 108 dB SPL. (c) IHC—base: Little IHC loss except at 108 dB SPL. (d) IHC—apex: Little IHC loss. Abscissa value [(a)–(d), here and in Figs. 4 and 5] from Eq. (2), basal OHC in (a). Without +15 dB SPL for ear-canal resonance. Symbol legend in (d) specifies exposure levels [see Table I(a)] and applies to (a)–(d) here and in Figs. 4 and 5. Gaussian bivariate ellipses at 0.95 probability level. Data from 59 chinchillas.

paring Fig. 3(a) with Fig. 2(a). The slope of the relation (a) was much less than would be predicted by the equal-energy hypothesis and the negative intercept (k) indicated that hair-cell loss did not begin until total energy had reached a certain magnitude. At the 108 dB SPL level (circles), clusters 2 and 3 did not show a relation with total energy. Clusters 2 and 3 had a total energy greater than $2^{15}\,\mathrm{Pa}^2\,\mathrm{s}$. Figures 3(b), (c), and (d) show the OHC loss in the apical OC and the IHC loss in the basal and apical halves, respectively. OHC loss in the basal OC spread somewhat into the apical half at the 108 dB SPL level [Fig. 3(b)]. However, there was little OHC loss at lower exposure levels [Fig. 3(b)] and even less IHC loss throughout the OC except for some at the highest exposure level in the basal half [Figs. 3(c) and (d)].

For the 4 kHz OBN exposures, clusters for IHC loss in the basal half [Fig. 3(c)] and OHC loss and IHC loss in the apical half [Figs. 3(b) and (d)] depended upon the cluster assignment for OHC loss in the basal half [Fig. 3(a)]. This dependency was based upon the observation that 4 kHz OBN exposures primarily produce OHC loss (often focal) in the basal half. Thus, the position of the other points and ellipses was relative to OHC loss in the basal half. An independent cluster analysis for the latter hair cell types and regions would have made their clusters smaller and tighter. However, the relative connections of the individual ears to the dominant region of OHC loss and the variance therein would have been lost.

Figure 4 shows the data for the Chronic subset (incorporating post-exposure recovery time) with the same arrangement as in Fig. 3. Clearly, OHC loss in the basal OC substantially increased post-exposure [Fig. 4(a)], as indicated by the changes in the proportion of the sample in the three clusters. Cluster 3 contained 51% of the sample in the Chronic subset whereas it only contained 10% in the Acute subset. The slope of the relation for cluster 1 between OHC loss and post-exposure recovery time [coefficient b in Eq. (2)] was 0.976 (Pearson $r=0.532$). The effect of the scaling produced by coefficients a and b can be appreciated by comparing Fig. 4(a) with Fig. 2(b). For cluster 1, OHC loss in the apical half [Fig. 4(b)] and IHC loss in both apical and basal halves [Figs. 4(c) and (d)] showed little or no increase. IHC loss at the highest exposure level in the basal half for clusters 2 and 3 increased dramatically [Fig. 4(c)], as did the OHC and IHC loss at the highest exposure level in the apical half [Figs. 4(b) and (d)]. Thus, the majority of the hair-cell loss from the 4 kHz OBN occurred post-exposure.

Figure 5 shows the data for the OHC loss in the basal [Fig. 5(a)] and apical [Fig. 5(b)] halves of the OC for the Interrupted subset. Because no ears were exposed to interrupted noise at a high SPL, the data were treated as a single cluster. Thus, there was no information about the effect of rest on clusters 2 and 3. In the Interrupted subset, IHC loss [Figs. 5(c) and (d)] was minimal throughout the OC. The coefficient for rest (c) in Eq. (2) was -0.923 (Pearson $r=0.772$). The negative slope indicated that rest during the exposure was beneficial.

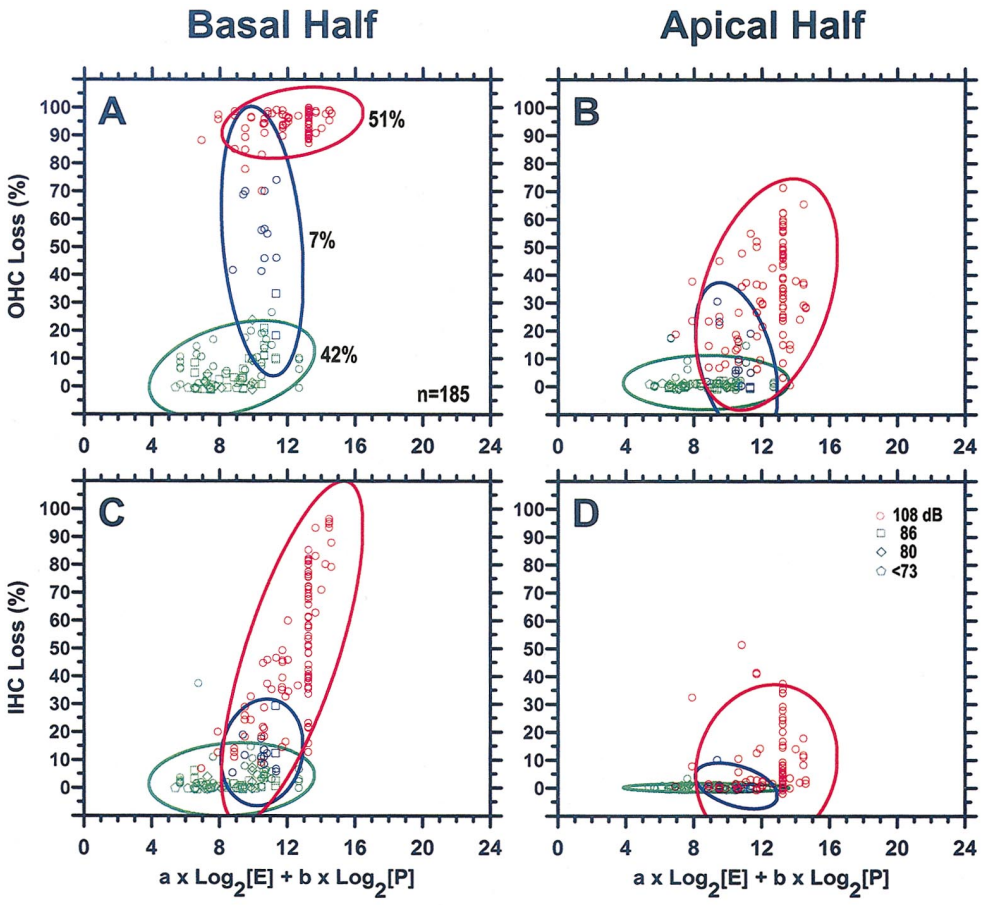

FIG. 4. 4 kHz OBN, chronic: (a) OHC—base: Strong relation with post-exposure recovery time; much more OHC loss with 108 dB SPL (circles). (b) OHC—apex: No increase in OHC loss (green) below 108 dB SPL; substantially more OHC loss with 108 dB SPL exposures. (c) IHC—base: Pattern much like OHC loss in apex. (d) IHC—apex: Pattern much like OHC loss in apex, but with much less IHC loss overall. Data from 128 chinchillas.

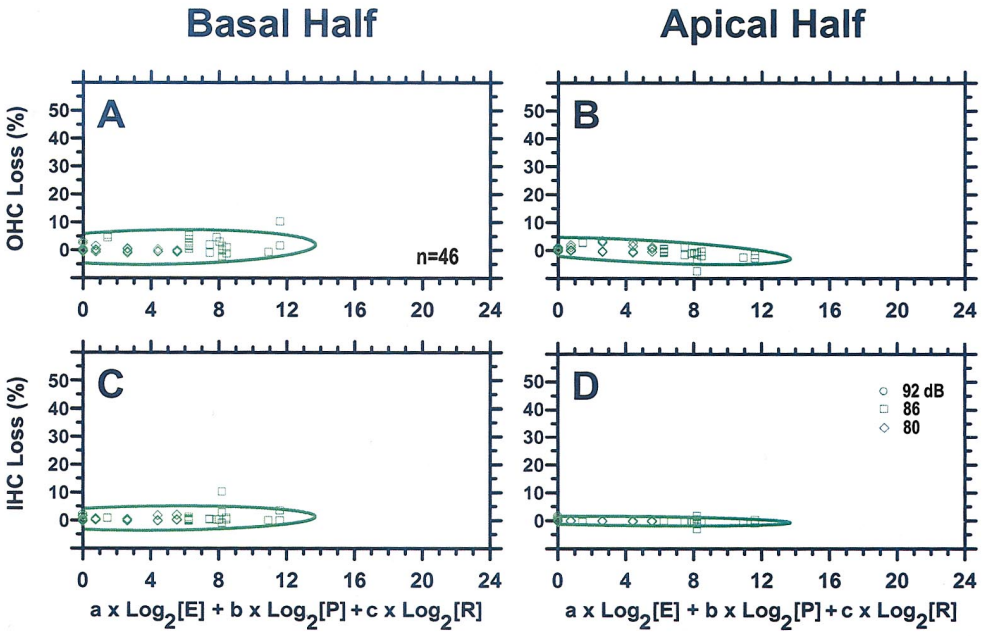

FIG. 5. 4 kHz OBN, Interrupted: (a)–(d) Hair-cell loss—base and apex: Minimal loss; in apex, somewhat less loss with longer rest periods. Exposures above 92 dB SPL not tested. Data from 30 chinchillas.

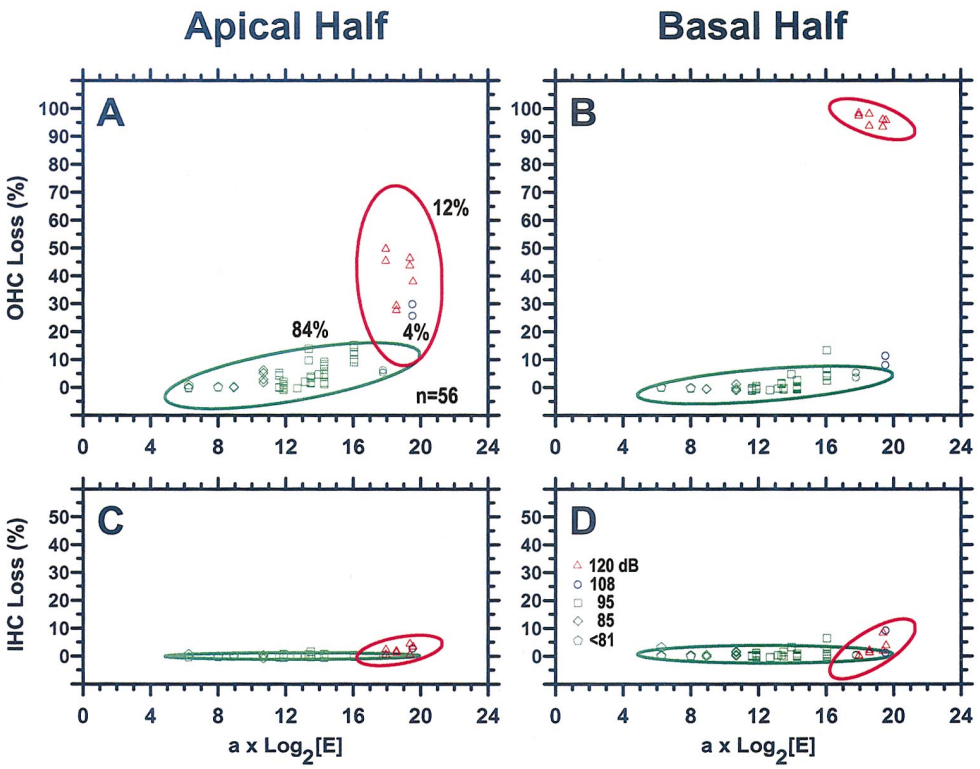

FIG. 6. 0.5 kHz OBN, Acute: (a) OHC—apex: Below 120 dB SPL, strong relation with *E*; 120 dB SPL is above critical level and shows no relation to *E*. Cluster 2 (blue) minimal and merged with cluster 3 (red). (b) OHC—base: Pattern much like basal OHC loss from the 4 kHz OBN, but at much higher *E*; cluster 2 merged with cluster 1 (green). (c) and (d) IHC—apex and base: Little IHC loss. Abscissa value [(a)–(d), here and in Figs. 7 and 8] from Eq. (2), apical OHC in (a). Symbol legend in (d) specifies exposure levels [see Table I(b)] and applies to (a)–(d) here and in Figs. 7 and 8. Gaussian bivariate ellipses at 0.95 probability level; ellipses for cluster 2 (blue) could not be drawn because the co-variance matrix was singular. Data from 37 chinchillas.

G. The 0.5 kHz OBN

The results for the 0.5 kHz OBN Acute, Chronic, and Interrupted subsets are shown in Figs. 6, 7, and 8, respectively, and in Table III. The symbols are as listed in Table I(b). Table III lists the number of ears and animals for each cluster of each subset, along with the means and standard deviations for the right-hand side of Eq. (2), and percent HCL for the apical OHC, IHC and basal OHC, IHC. The data are arranged as in Table II.

The OHC loss in the Acute subset for the apical half of the OC [Fig. 6(a)] showed a somewhat different pattern from that for OHC loss in the base with the 4 kHz OBN exposure [Fig. 3(a)]. There was a relation between OHC loss and total energy for cluster 1 but not clusters 2 and 3. Therefore, 120 dB SPL (triangles) was considered to be above critical level for this exposure. In cluster 1 (green), the slope of the relation for total energy in Eq. (2) was substantially larger ($a = 0.806$, $k = -8.302$, Pearson $r = 0.638$) than that for the 4 kHz OBN. In addition, cluster 2 (blue circles) was essentially missing and cluster 3 (red) appeared about where cluster 2 was with the 4 kHz OBN. Clusters 2 and 3 had a total energy greater than 2^{22} Pa^2 s. IHC loss was minimal throughout the OC [Figs. 6(c) and (d)]. The pattern of OHC loss in the basal half of the OC [Fig. 6(b)] was virtually indistinguishable from that seen with the 4 kHz OBN exposure [Fig. 3(a)], except that cluster 2 was minimally represented and more energy was required.

For 0.5 kHz OBN exposures, clusters for IHC loss in the apical half [Fig. 6(c)] and OHC and IHC loss in the basal half [Figs. 6(b) and 6(d)] depended upon the cluster assignment for OHC loss in the apical half [Fig. 6(a)]. This dependency was based upon the observation that 0.5 kHz OBN exposures primarily produce OHC loss (usually scattered) in the apical half. Thus, the position of the other points and ellipses was relative to OHC loss in the apical half.

In the Chronic subset illustrated in Fig. 7, the coefficient for recovery time in Eq. (2), cluster 1, was low ($b = 0.181$, Pearson $r = 0.173$). Thus, the vast majority of OHC loss in cluster 1 occurred during the exposure. OHC loss in cluster 3 increased post-exposure and cluster 2 appeared [Fig. 7(a)]. This secondary loss of hair cells included exposures at 95 dB SPL and higher. The relative contributions of the *E* and *P* terms was dominated by the *E* term and the adjustment from the *P* term was small. OHC loss in the basal half of the OC [Fig. 7(b)] showed a similar pattern to that from the 4 kHz OBN [Fig. 4(a)]. Much IHC loss appeared in both the apical [Fig. 7(c)] and basal [Fig. 7(d)] halves of the OC for clusters 2 and 3. However, there was little additional IHC loss in cluster 1.

The hair-cell loss for the Interrupted subset is shown in Fig. 8. Exposure levels greater than 101 dB SPL were not used, so the data were treated as two rather than three clusters. The coefficient for rest in Eq. (2) for cluster 1 was negative ($c = -0.435$, Pearson $r = 0.710$), again showing the benefit of rest during the exposure, albeit at about half the rate for the 4 kHz OBN exposures. However, cluster 2 was

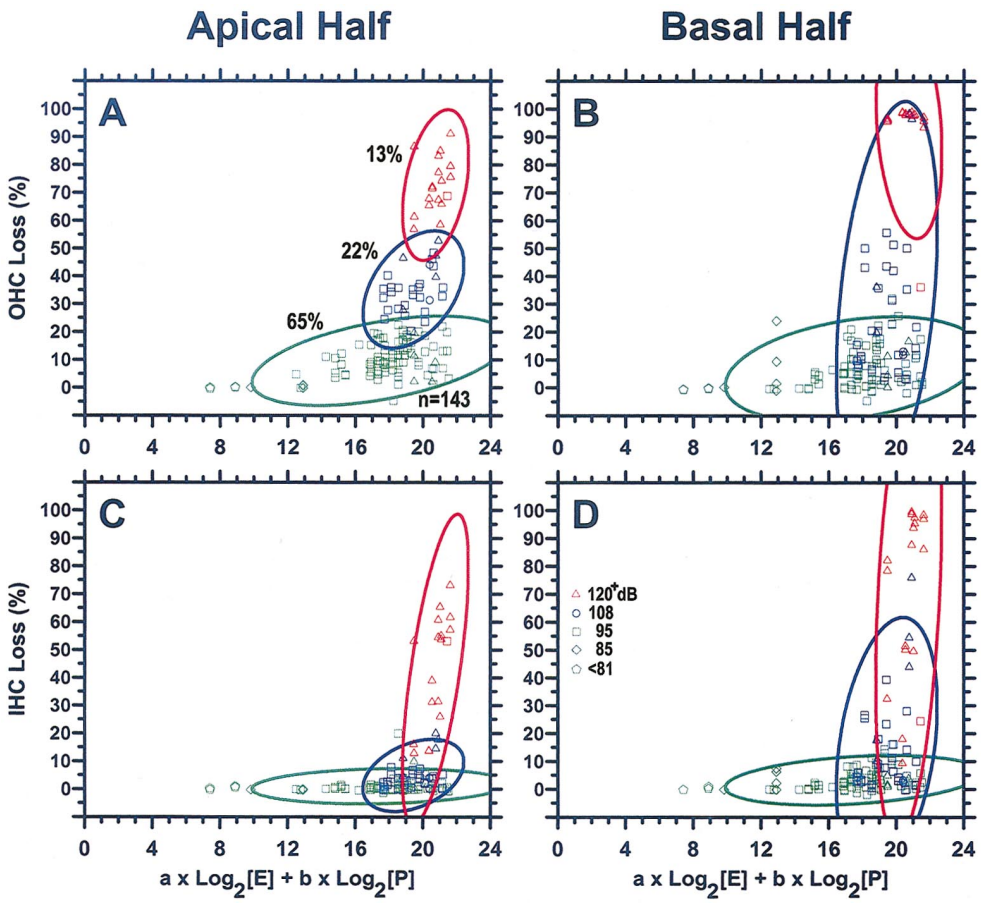

FIG. 7. 0.5 kHz OBN, Chronic: (a) OHC—apex: Poor relation with post-exposure recovery time; cluster 2 (blue) appears with exposures at 95 dB SPL and above; some increase in OHC loss with 120 and 128 dB SPL exposures (triangles). (b) OHC—base: Pattern much like Acute OHC loss in base from 4 kHz OBN. (c) IHC—Apex: Some increase in IHC loss (green) below 120 dB SPL; Much increased IHC loss at 120 and 128 dB SPL. (d) IHC—base: Much like IHC loss in apex. Data from 103 chinchillas.

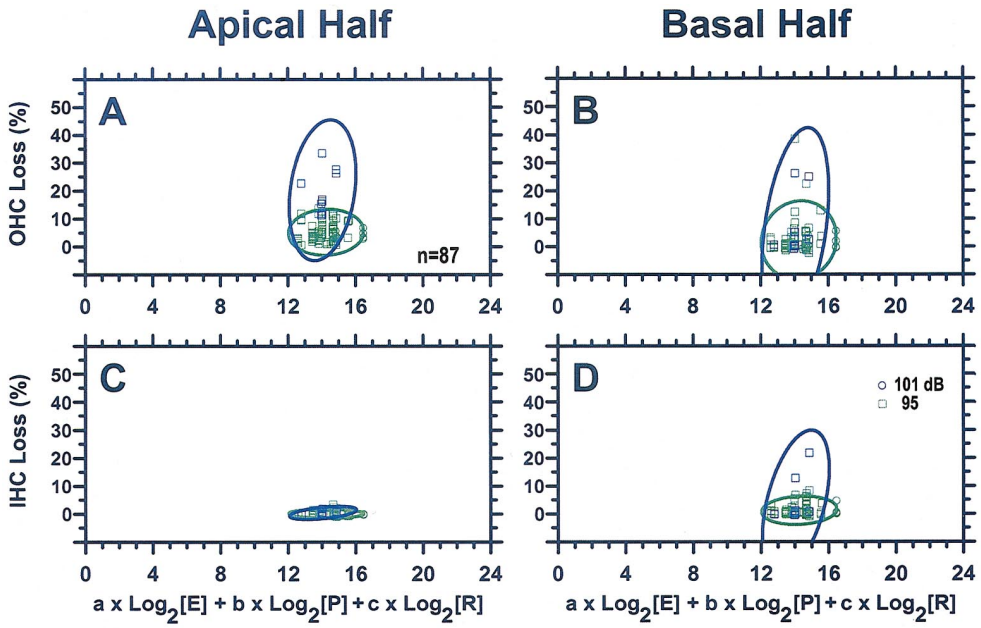

FIG. 8. 0.5 kHz OBN, Interrupted: (a) and (b) OHC—apex and base: Minimal OHC loss, but cluster 2 (blue) evident; slightly less OHC loss with longer rest periods. (c) IHC—apex: Minimal IHC loss. (d) IHC—base: Pattern much like OHC loss in apex and base. Exposures above 101 dB SPL not tested. Data from 59 chinchillas.

TABLE II. 4 kHz OBN, number of ears, percentages, and mean and standard deviation ( ) for cluster centers.

	No. Ears (%) [animals]	Energy+recovery+ rest	Basal OHC	Basal IHC	Apical OHC	Apical IHC
Acute subset						
Cluster 1	60 (67) [37]	5.56 (1.42)	1.62 (3.25)	0.67 (1.46)	−0.04 (0.92)	0.02 (0.55)
Cluster 2	21 (23) [15]	6.98 (0.26)	27.04 (12.88)	2.15 (1.90)	1.60 (2.52)	−0.04 (0.35)
Cluster 3	9 (10) [7]	7.90 (0.69)	91.92 (4.93)	8.49 (8.23)	18.55 (14.15)	0.54 (1.03)
Total:	90 (100) [59]					
Chronic subset						
Cluster 1	78 (42) [58]	8.73 (1.95)	6.25 (6.88)	2.97 (5.20)	1.48 (3.88)	0.05 (0.61)
Cluster 2	13 (7) [9]	10.52 (0.82)	51.97 (16.39)	12.60 (6.47)	8.79 (9.67)	0.78 (2.78)
Cluster 3	94 (51) [61]	12.31 (1.67)	94.50 (5.11)	48.69 (24.49)	33.12 (16.57)	8.00 (11.65)
Total:	185 (100) [128]					
Interrupted subset						
Cluster 1	46 (100) [30]	14.55 (2.79)	0.92 (2.45)	0.64 (1.71)	−0.36 (1.91)	−0.30 (0.69)
Total:	46 (100) [30]					

evident here, whereas it was not with the 4 kHz OBN. This suggests that critical level for the 0.5 kHz OBN exposure was about 95 dB SPL in chinchillas. In all of the Acute, Chronic, and Interrupted subsets, equivalent hair-cell loss from the 0.5 kHz OBN required more total energy than that for the 4 kHz OBN.

H. Specific doubling rates

Table IV lists the coefficients (doubling rates) determined from the model [Eq. (2)] for hair-cell loss versus total energy, recovery time, and rest for cluster 1. This hair-cell loss was primarily produced by noise exposures that were

TABLE III. 0.5 kHz OBN, number of ears, percentages, and mean and standard deviation ( ) for cluster centers.

	No. Ears (%) [animals]	Energy+recovery+rest	Apical OHC	Apical IHC	Basal OHC	Basal IHC
Acute subset						
Cluster 1	47 (84) [32]	12.42 (2.95)	4.12 (4.63)	0.04 (0.46)	0.75 (2.66)	0.58 (1.23)
Cluster 3	9 (16) [5]	18.93 (0.68)	37.36 (9.33)	2.24 (1.30)	76.87 (38.22)	3.14 (3.38)
Total:	56 (100) [37]					
Chronic subset						
Cluster 1	94 (65) [68]	17.47 (3.03)	9.51 (6.42)	0.86 (2.53)	6.70 (7.48)	3.10 (3.56)
Cluster 2	31 (22) [22]	19.45 (1.14)	35.11 (8.04)	4.76 (4.93)	31.36 (27.19)	15.13 (17.72)
Cluster 3	18 (13) [13]	20.76 (0.71)	72.71 (9.76)	42.91 (20.07)	93.99 (14.54)	69.50 (31.64)
Total:	143 (100) [103]					
Interrupted subset						
Cluster 1	78 (90) [53]	13.56 (1.42)	5.13 (3.29)	0.10 (0.65)	2.07 (5.61)	1.03 (2.02)
Cluster 2	9 (10) [6]	13.54 (1.03)	20.18 (7.67)	0.39 (0.71)	6.51 (10.91)	3.91 (7.85)
Total	87 (100) [59]					

TABLE IV. Model coefficients and Pearson *r* values ( ) for 4 and 0.5 kHz OBN, cluster 1.

4 kHz OBN	*N*	Coeff	Basal OHC	Basal IHC	Apical OHC	Apical IHC
Acute	60	*a* =	0.345 (0.366)	0.066 (0.155)	0.025 (0.093)	−0.018 (0.116)
		k =	−3.046	−0.218	−0.375	0.266
Chronic	76[a]	*b* =	0.976 (0.532)	0.443 (0.329)	0.284 (0.282)	0.003 (0.023)
Interrupted	46	*c* =	−0.923 (0.772)	−0.276 (0.533)	−0.219 (0.427)	−0.041 (0.313)
0.5 kHz OBN	*N*	Coeff	Apical OHC	Apical IHC	Basal OHC	Basal IHC
Acute	47	*a* =	0.806 (0.638)	0.007 (0.053)	0.381 (0.526)	−0.007 (0.020)
		k =	−8.302	−0.062	−5.124	0.678
Chronic	78[a]	*b* =	0.181 (0.173)	0.154 (0.299)	0.528 (0.424)	0.579 (0.691)
Interrupted	78	*c* =	−0.435 (0.710)	−0.040 (0.347)	−0.127 (0.176)	0.139 (0.485)

[a]Some ears not included due to nonexistent log(0).

below critical level. The left column of regression coefficients was based upon the above-described analysis methods. The coefficients in the other columns to the right were determined in the same way, but the cluster assignments were based upon those for the left column. Thus, the latter were dependent rather than independent coefficients.

For the 4 kHz OBN below critical level, the rates of basal IHC loss and protective effects of rest were usually much smaller than for OHC loss. In general, the rates of OHC and IHC loss were greater during the post-exposure recovery period than during the exposure, particularly in the basal half. The rates for prevention of both OHC and IHC loss with rest were negative as expected and surprisingly large for OHCs in the base. The rates of primary and secondary OHC and IHC loss in the apical OC were negligible. However, the rates of protection from loss with rest were substantial, although not as great as in the basal OC.

For the 0.5 kHz OBN below critical level, the rate of OHC loss in the apical half was the greatest during the exposure and negligible post-exposure. The rate of protection from loss due to rest was much less than for the 4 kHz OBN. The rates of IHC loss in the apical half and protection from loss with rest were negligible. In the basal half, the rates of OHC and IHC loss were similar to that for the basal loss from the 4 kHz OBN. The rates of protection from loss with rest, however, were not nearly as great and the coefficient was positive for IHCs in the base. The latter indicates that, for basal IHCs, the rest schedules used were not protective.

Assuming a linear relation between doubling of total energy and the primary OHC loss shown here, the equal-energy hypothesis would predict that the values of coefficient *a* from the 4 and 0.5 kHz OBNs would be 10.8 and 2.2, respectively. This is clearly not the case for primary basal OHC loss with the 4 kHz OBN and apical OHC loss with the 0.5 kHz OBN. For both OBNs, OHC loss in the other half of the OC and IHC loss in general, the values of coefficient *a* were well below 1.0.

I. Other noise-induced pathology

Although not reported in detail here, the data were also encoded for OC wipeouts, focal lesions, and nerve fiber loss. All three of these were more common in the 4 kHz OBN subsets than in the 0.5 kHz OBN subsets. OC wipeouts were rare in the Acute subsets, common in the Chronic subsets, and uncommon in the Interrupted subsets. Focal lesions were common in the high-level Acute subsets, much more common in the Chronic subsets, and uncommon in the Interrupted subsets. Nerve fiber loss was associated with substantial IHC loss.

IV. DISCUSSION

A. Hair-cell loss due to aging

The hair-cell loss from aging was very close to that found in an earlier examination which included some of the same cochleae (Bohne *et al.*, 1990), although the regression analysis used here was slightly different. For OHC loss in the basal half of the OC, there appeared to be a slight increase in slope for animals greater than 10 yr of age. However, there were too few older ears to address this issue with any degree of confidence. The present data revealed a slight, but significantly greater loss of OHCs in the apical half compared to the basal half of the chinchilla OC. The reason for this difference is unknown. Higher OHC loss due to aging in the apex of the guinea pig OC has been reported (e.g., Coleman, 1976; Ingham *et al.*, 1999). There is some evidence that these observations might apply to humans (Felder and Schrott-Fischer, 1995). Because the chinchilla has a low rate of hair-cell loss with age, it can be used for long-term noise-exposure studies without having an interaction between noise-induced hair-cell loss and age-related hair-cell loss.

B. Model of noise-induced hair-cell loss

The model for noise-induced hair-cell loss from below-critical-level exposures [Eq. (2)] was constructed as a convenient way to account for related issues and to analyze the cell-loss data. The coefficients are relative to the scaling of the independent variables. Thus, the specific values can only be interpreted with respect to that scaling. A change of scale would require adjusting their magnitudes accordingly. Because the scales for total energy, recovery time, and rest differ, the magnitudes of the coefficients cannot be interpreted relative to each other. In addition, the clustering was dependent upon OHC loss in the base for cochleae exposed to the 4 kHz OBN and in the apex for cochleae exposed to the 0.5 kHz OBN. The coefficients for OHC loss in the other half of the OC and IHC loss in both halves reflect this dependency.

The values of the coefficients would be different with an independent cluster analysis by cell type and OC region.

C. Susceptibility to noise-induced hair-cell loss

The data from moderate-level exposures (nearly all of cluster 1) suggests that all chinchillas were susceptible to noise-induced hair-cell loss during the exposure, but at a low rate. However, above-critical-level exposures produced a much wider range of hair-cell loss. Most animals were also susceptible to these exposures (cluster 2), but some were hypersensitive (cluster 3), while others were resistant (cluster 1).

The values for constant k from the regressions in the Acute groups, cluster 1 for both OBNs, agree with findings in the literature from Mills (1973) involving a 4 kHz OBN and Carder and Miller (1972) involving a 0.5 kHz OBN. Mills determined by extrapolation that the SPL of a 4 kHz OBN had to be greater than 47 dB before a TTS appeared. Also by extrapolation, Carder and Miller indicated that with a 0.5 kHz OBN, the SPL would have to 65 dB before there was a detectable TTS. It is interesting that the difference between the latter two levels is within 3 dB of the added pressure at the eardrum due to ear-canal resonance around 4 kHz. Bohne (1976) determined that continuous 4 kHz OBN exposures at and below 72 dB SPL for 2 or 9 d produced minimal sensory cell loss. For a 0.5 kHz OBN at 75 dB SPL, there was no significant sensory cell loss at 2 d, but about 10% OHC loss in the apex at 9 d.

D. Patterns of hair-cell loss during 4 kHz OBN vs 0.5 kHz OBN and critical level

From the data presented here, it is difficult to determine the exact magnitude of critical level. With the exception of two animals exposed at 92 dB SPL in the 4 kHz OBN Interrupted subset, animals were not exposed at levels between 86 and 108 dB SPL. Thus, critical level for the 4 kHz OBN exposure can only be estimated from the present data. However, the critical level must account for higher energy delivered to the cochlea due to ear canal resonance. Therefore, it is likely that the critical level for the 4 kHz OBN in the chinchilla is not far above 86 dB SPL, perhaps about 90 dB SPL.

For the 0.5 kHz OBN, only four animals in the Interrupted subset were exposed at a level between 95 and 108 dB SPL. All of these eight ears exposed at 101 dB SPL were in cluster 1. The 120 dB SPL level is clearly above critical level and one of two animals exposed at 108 dB SPL clustered with those at 120 dB SPL, indicating that 108 dB SPL is probably above critical level as well. The appearance of cluster 2 in the 95 dB SPL interrupted exposures suggests that the critical level for the 0.5 kHz OBN is below 95 dB SPL in chinchillas. Ward *et al.* (1981) exposed chinchillas for different durations to a 1.4 kHz OBN at different SPLs. These exposures should have put most of the damage in the middle of the chinchilla OC, overlapping both the apical and basal halves. Ward *et al.* found a substantial increase in the total percentage of OHC loss between exposures at 111 and 120 dB SPL after 30 d of recovery. Based on these results, Clark (1991) suggested that in chinchillas, critical level for continuous OBN exposures is about 115 dB SPL. The data reported here indicate that in chinchillas, 115 dB SPL for a continuous OBN is much too high for our broadened definition of critical level.

Bohne (1976) showed that a continuous, 4 kHz OBN exposure of 80 or 86 dB SPL or a 0.5 kHz OBN exposure of 85 dB SPL or greater for 9 d produced substantial hair-cell loss in focal regions in the basal half of the chinchilla OC. These observations are consistent with the results shown here in that clusters 2 and 3 did not appear, regardless of exposure level, until total energy had reached a certain magnitude. Thus, critical level is dependent upon both exposure level and duration. However, for clusters 2 and 3, the 2^7 Pa^2 s difference in onset of hair-cell loss between the 4 and 0.5 kHz OBNs is not accounted for by a 2^5 Pa^2 correction for 15 dB SPL of ear-canal resonance. Therefore, as observed by Bohne and Clark (1982), the base appears to be more sensitive to above-critical-level exposures than the apex.

A number of studies have been conducted in chinchillas to examine the equal-energy hypothesis with respect to broad-band impact noise (e.g., Henderson *et al.*, 1982; 1991; Roberto *et al.*, 1985; Levine *et al.*, 1998). Roberto *et al.* found exposures at and below a peak level of 119 dB SPL produced hearing loss and hair-cell loss that were consistent with the equal-energy hypothesis. However, above 119 dB SPL, hair-cell loss increased significantly. Levine *et al.* found that a peak level of 125 dB SPL was just below critical level for hearing loss. Henderson *et al.* (1982) found that with several exposures having equal energy, their animals did not develop the same amount of threshold shift as would be predicted by the equal-energy hypothesis. Rather, hearing loss increased with peak level. Using peak levels from 107 to 137 dB SPL, Henderson *et al.* (1991) found that their results did not conform to the equal-energy hypothesis in that both hearing loss and hair-cell loss increased more than would be predicted when peak levels were above 119 dB SPL.

Studies have been conducted in chinchillas to compare the consequences of OBN and broad-band impact noise (e.g., Ahroon *et al.*, 1993; Hamernik *et al.*, 2003). Ahroon *et al.* found that exposures that resulted in less than 10 dB PTS or less than 5% total hair-cell loss tended to produce effects that were consistent with the equal-energy hypothesis. On the other hand, the data from higher peak-level exposures were not consistent with the equal-energy hypothesis. There appeared to be an abrupt transition in hair-cell and hearing loss once a certain exposure level was exceeded. Hamernik *et al.* exposed chinchillas to continuous and interrupted OBN exposures at 85–99 dB SPL, impact noise at peak levels of 115–129 dB SPL, and combinations of the two. They did not address the critical-level issue, but they stated that their results were not consistent with the equal-energy hypothesis.

E. Primary versus secondary hair-cell loss and critical level

Bohne and Harding (2000) found that most of the OHC loss in the base occurs post-exposure from a 4 kHz OBN presented above critical level. One cause of hair-cell loss in

the noise-damaged OC is intermixing of cochlear fluids through the damaged reticular lamina (Ahmad *et al.*, 2003). Until the results of the present study, it was not clear that secondary hair-cell loss dominates the loss from 4 kHz OBN exposures below critical level. It was somewhat unexpected that most of the OHC loss from a 0.5 kHz OBN below critical level occurred during the exposure. However, it has been clearly shown that the patterns of hair-cell loss in the apex and base are different (e.g., Bohne and Harding, 2000).

Some researchers speculated that critical level represents a transition from noise-induced hair-cell loss to mechanical damage or acoustic trauma (e.g., Ward, 1981; Levine *et al.*, 1998). The classic definition of ''critical level'' is the intensity that produces irreversible damage in the OC no matter how brief the exposure. This level is generally thought to represent the ''elastic limit'' that, when exceeded, results in immediate rupture of the OC. However, the primary loss in clusters 2 and 3 appeared at exposure levels well below those commonly associated with mechanical damage and the secondary loss in these clusters increased substantially during post-exposure recovery. These results suggest that the definition of critical level should not be limited to the threshold for mechanical damage. The definition of critical level should be expanded to include the level at which substantial secondary hair-cell loss occurs post-exposure.

F. Rest during the exposure and critical level

In the present study, rest during moderate-level exposures was protective for hair-cell loss; more for the 4 kHz OBN than the 0.5 kHz OBN. Bohne *et al.* (1985; 1987) and Clark *et al.* (1987) examined the effect of rest during the exposure on hearing loss and hair-cell loss. They found that interrupted exposures produced less TTS and PTS and less hair-cell loss than continuous exposures with equal energy. Ward (1991) examined interrupted exposures with equal energy. He found that rest during the exposure reduced OHC loss more than would be predicted by the equal-energy hypothesis. In a large study using narrow-band impact noise, Hamernik and Ahroon (1998) compared continuous and interrupted exposures with equal energy. They found that rest produced a slightly lower PTS but no difference in sensory-cell loss. Dolan *et al.* (1976) reported that with very high level exposures, rest was not protective. The equal-energy hypothesis predicts that continuous and interrupted exposures with equal total energy would produce the same amount of hair-cell loss. Thus, the results reported here do not support the equal-energy hypothesis.

G. Other species

Examinations of the equal-energy hypothesis have also been conducted in species other than chinchillas. Eldredge and Covell (1958), Salt *et al.* (1981), Goulios and Robertson (1983), and Fredelius *et al.* (1987) looked at this issue in guinea pigs. Eldredge and Covell introduced the equal-energy hypothesis from their results. However, none of the latter three studies produced results that were consistent with the assumptions of the equal-energy hypothesis. The critical level appeared to be about 20 dB SPL higher in the guinea pig compared to the chinchilla (e.g., Eldredge and Covell, 1958). Borg and Engström (1989) looked at the equal-energy hypothesis in rabbits using a 4 kHz OBN at either 85 or 115 dB SPL. They found results similar to those reported for chinchillas. Bies and Hansen (1990) conducted a retrospective study of a large human population. They found that the equal-energy hypothesis was not adequately supported. They suggested that NIHL scales better on the integral of pressure rather than pressure squared. However, this conclusion may have been the result of fitting data from a mixture of both above- and below-critical-level exposures.

H. Critical level in humans

Chinchillas are about 10 dB SPL more sensitive to noise than humans (Eldredge *et al.*, 1973). Thus, critical level in humans is expected to be about 10 dB SPL higher than predicted for chinchillas. Also, humans tend to be exposed to noise on an interrupted schedule. The frequency spectrum of the noises to which humans are exposed varies among a number of sources. These spectrums effect the base and apex differently with low-frequency noise causing hair-cell loss in both the apex and base (e.g., Bohne and Harding, 2000). The observations reported here along with those in the literature indicate that critical level is different depending upon the characteristics of the noise exposure and the location in the OC that the noise has its greatest effect. In addition, critical level differs with continuous and interrupted exposures. Also, critical level appears to be species specific.

V. CONCLUDING REMARKS

The principal results from this study are as follows.

(a) Primary hair-cell loss is related to total energy of the exposure, provided that the intensity of the exposure is below critical level. However, the specific doubling rates are not consistent with the equal-energy hypothesis. In addition, the magnitude of the relation is noise frequency-spectrum dependent.

(b) The definition of critical level should include the noise intensity at which a substantial amount of secondary hair-cell loss occurs post-exposure. Critical level is species specific and depends upon the frequency spectrum of the noise and the ear-canal resonance of the animal and its susceptibility to noise.

(c) With exposures below critical level, periods of rest during the exposure substantially reduce OHC and IHC loss for the 4 kHz OBN, less so for the 0.5 kHz OBN.

(d) With a 4 kHz OBN, much OHC loss occurs post-exposure. With a 0.5 kHz OBN below critical level, much OHC loss occurs during the exposure while much of the loss from above-critical-level exposures occurs post-exposure. For both OBNs, most IHC loss occurs post-exposure.

(e) Hair-cell loss in the base resulting from a 0.5 kHz OBN is strikingly similar to that due to a 4 kHz OBN, but requires more energy, in part due to the lack of ear-canal resonance at 0.5 kHz.

Nordmann *et al.* (2000) found that cochleae with TTS have a completely different histopathological appearance than those with PTS and concluded that TTS is partially protective against PTS. With a damaging noise exposure, much hair-cell loss occurs post-exposure. Thus, there is a window of opportunity to administer treatment to prevent or minimize noise-induced secondary hair-cell loss, and therefore, NIHL. However, such treatment should be directed at preventing hair-cell loss and PTS, not TTS.

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