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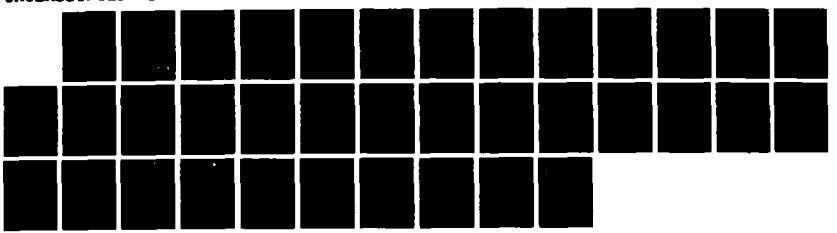

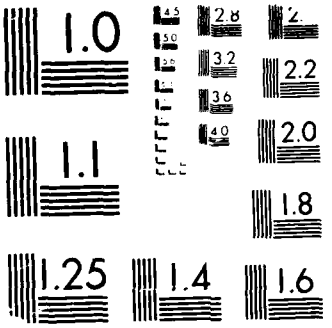

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REPORT NO. OTO-1-88

ASSESSMENT OF COCHLEAR DAMAGE AFTER MICROWAVE IRRADIATION

Final Report

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OBJECTIVE: To determine whether or not excessive exposure to microwaves results in permanent damage to the inner ear.

METHODS: A group of 15 chinchillas was exposed for one hour to pulsed microwaves (1250 MHz) of 20μsec duration and 0.1-Hz repetition rate, with a net peak power of 500 KW and an average power of one Watt at the WRAIR Microwave Laboratory, Washington, D.C. Seven animals were sham-exposed for one hour using the same apparatus and sedation. For the sham exposures, the microwave equipment was powered but no radiation was delivered. The cochleas from 20 control chinchillas of the same age range as the animals in the microwave study were available for comparison purposes. The controls had spent their entire lives in sound-treated animal quarters at Washington University in St. Louis, MO. (Continued)

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The cochleas from all animals were processed for histological evaluation as plastic-embedded flat preparations. Some animals were processed less than 24 hours after their exposures; the rest were processed after a month or more of recovery.

In each cochlea, the following quantitative data were obtained: the extent and pattern of degeneration in the sensory cell populations; the number of missing pillar cells; the extent and location of degeneration of the stria vascularis and of the myelinated nerve fibers in the osseous spiral lamina.

CONCLUSION: Several different patterns of cochlear damage were found in the sham- and microwave-exposed cochleas including: loss of outer hair cells scattered over a broad region of the low-frequency (apical) portion of the organ of Corti; narrow lesions of severe loss of inner and/or outer hair cells in the high-frequency (basal) portion; degeneration of part of the stria vascularis.

Based on quantitative and statistical differences between the microwave ears and those damaged by excessive exposure to noise, it is highly unlikely that the damage found in the microwave study is the result of exposure to environmental noise. Review of the data from the control chinchillas indicates that it is also unlikely that the damage in the microwave cochleas was preexisting. On the other hand, in view of the similarities between the cochlear lesions in the sham- and microwave-exposed animals, the damage cannot be attributed solely to exposure to microwaves. It is concluded that some unidentified ototraumatic agent at WRAIR Microwave Laboratory was responsible for the cochlear damage found in the present study. Additional studies must be conducted in order to identify the causative agent(s) and to determine the potential hazard of exposure to microwaves.

SUMMARY

ASSESSMENT OF COCHLEAR DAMAGE AFTER MICROWAVE IRRADIATION

OBJECTIVE: To determine whether or not excessive exposure to microwaves results in permanent damage to the inner ear.

METHODS: A group of 15 chinchillas was exposed for one hour to pulsed microwaves (1250 MHz) of 20 usec duration and 0.1-Hz repetition rate and an average power of 1 Watt. The specific absorption rate of various measurement sites in the head ranged from 2-8 W/kg. The exposures were done at the WRAIR Microwave Laboratory, Washington, D.C. Seven animals were sham-exposed for one hour using the same apparatus and sedation. For the sham exposures, the microwave equipment was powered but no radiation was delivered. The cochleas from 20 control chinchillas of the same age range as the animals in the present study were available for comparison purposes. The controls had spent their entire lives in sound-treated animal quarters at Washington University in St. Louis, MO.

The cochleas from all animals were processed for histological evaluation as plastic-embedded flat preparations. Some animals were processed less than 24 hour after their exposures; the rest were processed after a month or more of recovery.

In each cochlea, the following quantitative data were obtained: the extent and pattern of degeneration in the sensory cell populations; the number of missing pillar cells; the extent and location of degeneration of the stria vascularis and of the myelinated nerve fibers in the osseous spiral lamina.

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FOREWORD

The authors are indebted to C.G.B. Campbell, M.D., Ph.D., WRAIR, and James C. Lin, Ph.D., University of Illinois, for their advice and assistance in performing the acoustic and microwave dosimetry measurements. Mr. Thomas J. Watkins, Washington University School of Medicine provided excellent technical assistance throughout the study.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (DHHS NIH Publication No. 85-23, Revised 1985).

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INTRODUCTION

The effects of microwaves on living organisms are of considerable interest to many individuals in industry and the military. Excessive exposure to microwaves in the frequency range of 10-40,000 MHz has been shown to have deleterious effects on humans and experimental animals. When the microwave exposure levels are sufficiently high to produce temperature increases of several degrees Celsius, adverse health effects result. These effects include: cataracts, corneal opacities, testicular degeneration and cardiovascular alterations (Cleary, 1973).

Exposure to pulsed microwaves of high peak power elicit auditory sensations described as clicking, buzzing or hissing, depending on the characteristics of the microwaves. The mechanism responsible for the auditory sensation is thought by some to be thermoelastic expansion (Chou and Guy, 1982). Although the possibility of auditory effects from microwave exposure have been known since the late 1940's, there has never been a study of the effects of exposure on the anatomy of the inner ear epithelium. Damaging effects may well be expected in view of the high equivalent sound pressure levels induced by microwaves such as those used for communication purposes.

In order to determine whether or not exposure to microwaves results in permanent damage to the inner ear, a pilot study was conducted. A divided group of young adult chinchillas was either exposed to microwaves or sham-exposed. The inner ears of the animals were prepared for histological examination shortly after completion of treatment or after a month of recovery. Details of the microwave exposures and histological findings are presented in this report.

MATERIALS AND METHODS

Subjects:

Twenty-two chinchillas ranging in age from 1.0 to 2.5 years without a prior history of exposure to ototraumatic agents were used for this study. All chinchillas were born and raised in sound-shielded animal quarters at Washington University in St. Louis. Fifteen chinchillas were exposed to microwaves and seven animals were sham exposed. Four additional animals were used for dosimetry and/or determination of the acoustical properties of the microwave exposure.

For their exposure to microwaves, the animals were air shipped from St. Louis in groups of four to Washington, D.C., and transported to the Forest Glen Annex of Walter Reed Army Institute of Research. After the four animals had been treated, they were air shipped back to St. Louis. The cochleas from two shams and four exposed chinchillas were processed for histological examination within 24 hours after completion of treatment (these are termed "acute" in the remainder of this report). The cochleas from four shams and 11 exposed chinchillas were processed after a month or more of recovery (these are termed "chronic" in the remainder of this report). One sham died in the exposure apparatus of undetermined causes.

Microwave Exposure:

Apparatus:

Details of the microwave exposure system can be found in Bassen et al (1988). Briefly, the exposure apparatus consisted of a modified Klystron microwave amplifier with a 30.48-m long waveguide (WR650) and an animal-exposure cavity. The modifications permitted the generation of pulsed microwaves with a maximum peak power of 1 MW. An 8-cm diameter hole was cut into the broadwall of the waveguide, approximately 30 cm from its copper-screen endplate. A rigid metal cylinder, mounted over the hole, was used to support the animal's body during the exposure. A foam plastic block was placed in the waveguide to support the animal's head during the exposure.

Exposure:

Prior to the exposure, each animal was injected with Ketamine HCl (40 mg/kg IM) and Acepromazine (0.5 mg/kg IM). After sedation, the animal's head was placed in the waveguide with the ventral surface resting on the foam block and its body supported by the metal cylinder. About half of the animals remained virtually motionless throughout their one-hour exposure without supplemental medication. The rest were given another dose of Ketamine (20 mg/kg IM) after 30 minutes of treatment, when jaw and head motion were observed.

TABLE 1: CHINCHILLAS USED FOR PILOT MICROWAVE STUDY

EAR #	AGE (YRS)	TREATMENT	RECOVERY
690	1.4	1 hr MW	Acute
691	1.2	1 hr MW	Acute
695	1.0	1 hr MW	Acute
696	1.3	Sham exp	Acute
703	1.3	1 hr MW	Chronic
704	1.3	1 hr MW	Chronic
701	1.5	Sham exp	Acute
702	1.5	1 hr MW	Acute
706	1.4	1 hr MW	Chronic
707	2.0	1 hr MW	Chronic
711	1.8	1 hr MW	Chronic
712	1.8	1 hr MW	Chronic
713	1.9	Sham exp	Chronic
714	2.5	Sham exp	Chronic
715	1.6	1 hr MW	Chronic
716	1.6	Sham exp	Chronic
717	1.5	1 hr MW	Chronic
718	1.3	1 hr MW	Chronic
719	1.3	1 hr MW	Chronic
-	1.3	Sham exp	Died
720	1.5	Sham exp	Chronic
721	1.4	1 hr MW	Chronic

Fifteen animals were exposed for one hour each to pulsed microwaves (1250 MHz) of 20-usec duration and 0.1-Hz repetition rate, with a net peak power of 500 KW and an average power of one Watt. Seven animals were sham exposed for one hour each using the same apparatus and sedation. For the sham exposures, the microwave amplifier was powered but no radiation was delivered. The first 10 animals in the study (see Table 1) were oriented with their left ears toward the microwave source while the last 11 animals had their right ears toward the source.

Microwave Dosimetry and Thermography:

The heads of two intact chinchilla cadavers were placed in the waveguide and irradiated for 10-15 seconds with 1250-MHz microwaves having an average power of 100 Watts. One non-perturbing Luxtron fiberoptic thermometry probe (1 mm diameter) was placed at various locations in the brain and one probe was sutured into the cochlea facing the incident radiation. One cadaver was encapsulated in urethane foam and split along mid-line and evaluated with a thermographic camera immediately after irradiation, to reveal microwave-dose distribution throughout the head. These procedures permitted the determination of the spatial distribution of the rate of absorbed microwave energy [specific absorption rate (SAR)] throughout the brain as well as providing precise SAR data for the cochlea. In order to determine the amount of heating which would occur during constant irradiation, one cadaver and one anesthetized chinchilla (Nembutal - 50 mg/kg) were each irradiated for several minutes (using an average power of 1 Watt). Temperature was measured in the colon of the anesthetized animal during treatment.

Acoustic pressure level of microwave exposures:

A broadband (i.e. 1 kHz to 2 MHz), omnidirectional PZT needle hydrophone was used to determine the transient acoustic pressures which were generated in the chinchilla's head during the microwave exposure. The performance of the hydrophone was determined before use by immersion in various sized water vessels that were placed in the waveguide exposure cavity and exposed to 400-KW peak power microwave pulses. The recorded waveforms were damped sinusoids that were of the expected frequency, with amplitudes which were inversely proportional to the size of the water vessel. This result ensured that no distortion or long-term artifact was induced in the hydrophone or its associated electronics by exposure to intense microwave pulses.

The hydrophone was then inserted into the brain of an anesthetized chinchilla through a 3-mm hole in its skull and was oriented perpendicular to the electric field vector to minimize the hydrophone's interaction with the microwave field. Acoustic sound pressure levels induced by exposure to 20-usec microwave pulses with 200- and 460-KW peak power were then recorded with transient data acquisition/storage instruments (Nicolet).

Histological Processing:

At the appropriate recovery time, each animal was anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body weight). The animal was secured to a slant board, its chest opened and the aorta cannulated through the left ventricle. The vascular system was flushed for two minutes with Dalton's buffer (pH: 7.2-7.4; temp: 37 ^{o}C), followed by perfusion for 3-7 minutes with 2 or 4% glutaraldehyde, 0.1% Malachite green in Dalton's buffer. After termination of the vascular perfusion, the animal was decapitated, its temporal bones removed, and its cochleas perfused through scala tympani with cold 1% osmium tetroxide (OsO_4) in Dalton's buffer for five minutes. The specimens were then immersed in a large volume of 1% OsO_4 in Dalton's buffer for two additional hours at 4 ^{o}C.

After fixation was completed, the specimens were washed in Tyrode's solution, dehydrated in a graded series of ethanol and propylene oxide and gradually infiltrated with araldite. After the araldite polymerized, the cochlear bone was removed with a sharpened steel pick, and all segments of the cochlear duct were removed from the specimen using small pieces of double-edged razor blades. The segments of OC from all cochleas were trimmed parallel to the basilar membrane, reembedded in a thin layer of araldite and examined as plastic-embedded whole mounts by phase contrast microscopy (Wild M20). Full details of this technique have been published previously (Bohne, 1972).

Microscopic Evaluation:

All specimens were evaluated in the same fashion. The lengths of the segments of the OC from each cochlea were measured (Bohne et al, 1986). Counts of missing inner hair cells (IHCs), outer hair cells (OHCs), inner pillars (IPs) and outer pillars (OPs) were made.

In order to summarize the data on sensory cell loss, the following strategy was adopted. In segments of the OC in which the sensory cell loss was scattered, the length of the segment was used as the bin width and the percentage of missing IHCs and OHCs in the bin was calculated. When cell loss was concentrated within a portion of a segment, those area(s) were localized by arithmetically subdividing the segment on the basis of the IHC or OHC counts. The percentage of missing hair cells was then calculated for each of the arithmetic subdivisions (Bohne et al, 1986).

Lesions in the basal turn of the OC in which 50% or more of the sensory cells are missing over a distance of about 0.04 mm have been termed high-frequency lesions (HFLs) (Bohne and Clark, 1982). These lesions were classified as IHC HFLs, OHC HFLs or combined HFLs, when IHCs, OHCs or both IHCs and OHCs, respectively, were missing in numbers equal to or greater than 50% (Bohne et al, 1987).

Regions of loss of myelinated nerve fibers (MNFs) were located by noting the portions of the osseous spiral lamina (OSL) which were lightly stained with the OsO_4 fixative. The percentage of MNF loss was estimated by visually comparing the staining intensity in the damaged area to that in a control cochlea at a comparable location (Bohne et al, 1985). This technique is capable of detecting regions of concentrated loss of MNFs. However, it cannot detect a widespread, low-level loss of fibers.

Areas of degeneration of the stria vascularis (SV) appeared as unstained portions of the stria vascularis/spiral ligament complex (Fried et al, 1976). The lengths of these regions were measured and their apical-basal positions were determined.

For each ear, the data on sensory cell loss were used to prepare a cytocochleogram, a graph depicting the percentage of missing IHCs and OHCs as a function of percentage distance from the apex of the OC. The approximate frequency-place map on the cytocochleogram is based on the formula developed by Eldredge et al (1981). The information on the location of SV degeneration and the extent and location of MNF degeneration were also plotted on the cytocochleograms. The cytocochleograms from the right and left ears of all sham- and microwave-exposed animals were visually compared in order to determine whether or not the pattern of damage was symmetrical in the two ears.

For all cochleas, the following statistics summarizing the percentage of IHC and OHC losses were calculated: 1) loss averaged over the entire OC; 2) loss in the low-frequency region (15-35% distance from apex); and 3) loss in the high-frequency region (60-90% distance from apex). In addition, for each cochlea with one or more HFLs, the summed size of the lesioned area was determined by adding together the length(s) of the individual lesions. These statistics allowed easy comparison of the damage among the sham-exposed and microwave-exposed animals and control chinchillas of comparable ages.

RESULTS

Dosimetry:

Table 2 shows the results of the SAR determinations in the cadavers as calculated from the initial rate of rise of temperature and the estimated values of specific heat for the various tissues of interest. Since the experimental chinchillas were exposed under conditions which were similar to the cadaver exposures, the data in Table 2 should be valid within ± 20% for any chinchilla so exposed. The average SAR for the ear closest to the source may have been 2-3 times larger than that of the opposite ear. It was found that during the exposure, most of the microwave energy was absorbed by the head of the chinchilla. No leakage was detected at the endplate of the waveguide or at the open end of the body-support cylinder.

TABLE 2: SPECIFIC ABSORPTION RATE (SAR) FOR HEAD OF CHINCHILLA EXPOSED TO MICROWAVES*

LOCATION	SAR - AVERAGE (W/kg)	SAR - TEMPORAL PEAK (W/kg)
Brain - incident surface	5	2.5×10^6
Brain - center	3	1.5×10^6
Brain - opposite incident surface	2	1.0×10^6
Cochlea - interior	8	4.0×10^6

*Exposure parameters: 1250 MHz pulses, 20-usec duration; Net peak power - 500 KW; Average power - 1 Watt; Repetition rate - 0.1 Hz.

The temperature in the brain and cochlea of the cadaver which was irradiated continuously for several minutes never rose by more than 0.5 ^{o}C during the exposure. Temperature in the colon of the sedated, thermoregulating chinchilla remained constant. Thus, it is unlikely that any damage found in cochleas of the irradiated animals can be attributed to tissue heating.

Acoustic pressure of the microwaves:

A microwave-induced artifact was observed in the hydrophone's output during the short period (20 usec) of the pulse, but the damped sinusoidal waveform that was recorded continued

long after microwave-induced artifact had ended. The acoustic sound pressure level that was induced in the midbrain during irradiation had a fundamental frequency of approximately 60 kHz, and an exponential decay to 10% of its maximum amplitude after about five cycles. The maximum peak-to-peak amplitude of the pulses was 120×10^{-3} Bar (12×10^{4} dyne/cm^2) for 460 KW peak power microwave pulses and about 60×10^{-3} Bar (6×10^{4} dyne/cm^2) for 200 KW peak power pulses. These values correspond to sound pressure levels of 175.5 and 169.5 dB, respectively.

Cochlear damage in control chinchillas:

Twenty-three cochleas from 20 laboratory-raised chinchillas which ranged in age from 1.0 to 2.5 years were used as controls for determining the effects of exposure to transportation noise, to the stress from excessive handling etc. on the sham- and microwave-exposed animals. These specimens had already been prepared and examined microscopically as plastic-embedded whole mounts. The statistics calculated for sensory and supporting cell loss and SV and MNF degeneration obtained from the sham- and the microwave-exposed animals were also available for the cochleas from the control chinchillas.

In this group, the average percentage of missing sensory cells was as follows: IHCs - 0.4 ± 0.3%, OHCs - 0.9 ± 0.7% in the 0-100% region; IHCs - 0.4 ± 0.6%, OHCs 0.8 ± 0.5% in the 15-35% region; IHCs - 0.3 ± 0.3%, OHCs - 0.8 ± 1.4% in the 60-90% region. Pillar losses were minimal, averaging one missing IP and one missing OP throughout the OC. None of these ears had any areas of SV degeneration. Two of the 23 cochleas had small IHC lesions: a 1.5-year-old animal had a 0.03-mm lesion (i.e. 3 IHCs missing in a row) located at 19.2-19.4% distance from the apex and a 1.7-year-old animal had a 0.06-mm IHC lesion (i.e. 6 missing IHCs) located at 31.1-31.5% distance and an associated loss of MNFs. A cytocochleogram from a typical 1.7-year-old chinchilla is shown in Figure 1.

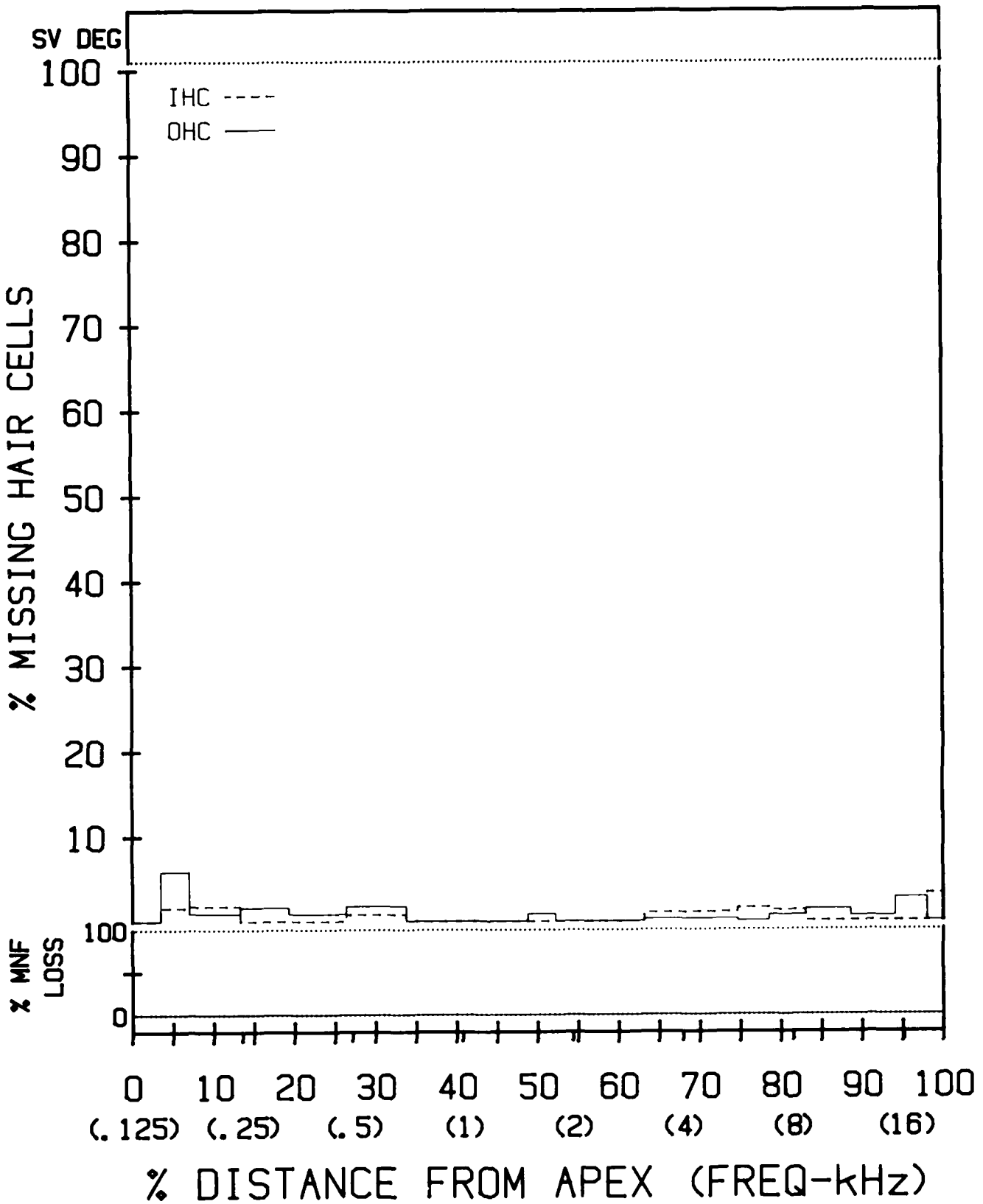

Figure 1: Typical cytocochleogram from a 1.7-year-old control chinchilla depicting the percentage of missing inner hair cells (IHC - dashed line) and outer hair cells (OHC - solid line) as a function of percentage distance from the apex of the OC. Although not present in this cochlea, graph can also accommodate information on the extent and location of degeneration of myelinated nerve fibers (% MNF LOSS) and stria vascularis (SV DEG).

Cochlear damage in sham-exposed chinchillas:

Of the 12 cochleas in this group, two acute and five chronic specimens (58%) sustained OHC losses in the 15-35% region (Table 3) which were more than three standard deviations (SDs) greater than the average loss in the control group. One acute and five chronic cochleas (50%) had a total of 12 HFLs which were located between 58% and 83.3% distance from the apex. Nine of the lesions (75%) were IHC HFLs and three lesions (25%) were combined HFLs (Table 4). All but two of the HFLs had an associated loss of MNFs (Table 4). For the individual cochleas with HFLs, the summed size of the lesioned area(s) ranged from 0.09 mm to 0.93 mm and averaged 0.64 mm.

Two of the shams (22%) had a region of SV degeneration: an acute specimen had a 0.69-mm lesion in the 60.4-63.1% area and a chronic specimen had a 2.38-mm lesion in the 1.4-11.8% area (Table 5). The cytocochleogram from the chronic, sham-exposed cochlea which had the largest OC lesion is shown in Figure 2.

In summary, cochlear damage was found in all six sham-exposed chinchillas in one or more of the following areas: low-frequency region of the OC, high-frequency region, SV. Four of the six animals (67%) had sizable differences between the pattern of damage in their right and left ears. However, there was no relation between amount of damage in a particular ear and its orientation with respect to the inactive microwave source.

TABLE 3: COCHLEAR DAMAGE IN SHAM-EXPOSED CHINCHILLAS (N=6)

	% MISSING HAIR CELLS		# MISSING PILLARS		SV DEG	MNF LOSS
EAR #	IHC (0-100%) (15-35%) (60-90%)	OHC	IP	OP	(Y/N)	(Y/N)
696R*	3.3 1.5 9.1	1.3 0.6 2.0	7	0	Y	Y
696L*	0.7 0.3 0.4	2.0 3.5 0.3	0	0	N	N
701R*	0.3 0.1 0.2	1.8 4.6 0.6	1	1	N	N
701L*	0.5 0.3 0.4	1.1 2.0 0.6	1	0	N	N

*Acute ears. Rest of shams are chronic.

TABLE 3 (CONT): COCHLEAR DAMAGE IN SHAM-EXPOSED CHINCHILLAS

EAR #	% MISSING HAIR CELLS IHC (0-100%) (15-35%) (60-90%)	% MISSING HAIR CELLS OHC	# MISSING PILLARS IP	# MISSING PILLARS OP	SV DEG (Y/N)	MNF LOSS (Y/N)
713R	3.5 0.3 10.6	1.5 1.0 2.2	13	9	Y	Y
713L	4.8 0.5 15.1	3.0 1.2 7.0	55	46	N	Y
714R	5.0 1.4 14.4	2.6 6.9 1.5	2	0	N	Y
714L	5.6 1.6 15.9	2.2 3.8 2.2	3	3	N	Y
716R	0.9 0.5 0.2	1.7 2.8 0.5	10	5	N	Y
716L	0.2 0.1 0.0	1.3 1.8 0.6	10	0	N	N
720R	0.3 0.6 0.0	2.7 7.7 0.5	0	1	N	N
720L	0.2 0.6 0.0	2.1 6.4 0.4	0	2	N	N

TABLE 4: SIZE AND TYPE OF HFLs IN SHAM-EXPOSED CHINCHILLAS WITH LESIONS

GROUP EAR #	HFL TYPE	EXTENT (mm)	% LOCATION	% MNF LOSS
ACUTE				
696R	IHC	0.65	74.1-77.5	---
	IHC	0.15	80.0-80.8	5
CHRONIC				
713R	COMBINED	0.66	69.5-73.2	30-98
713L	COMBINED	0.93	69.6-74.6	25-95
714R	IHC	0.11	75.5-76.0	30
	IHC	0.28	78.1-79.5	95
	IHC	0.31	81.7-83.3	---
714L	IHC	0.11	72.6-73.2	75-80
	IHC	0.43	76.4-78.8	99
	IHC	0.04	80.0-80.2	20
	IHC	0.08	80.5-80.9	40
716R	COMBINED	0.09	58.0-58.5	75

TABLE 5: DEGENERATION OF STRIA VASCULARIS IN SHAM-EXPOSED CHINCHILLAS WITH LESIONS

EAR #	EXTENT (mm)	% LOCATION
ACUTE		
696R	0.69	60.4-63.1
CHRONIC		
713R	2.38	1.4-11.8

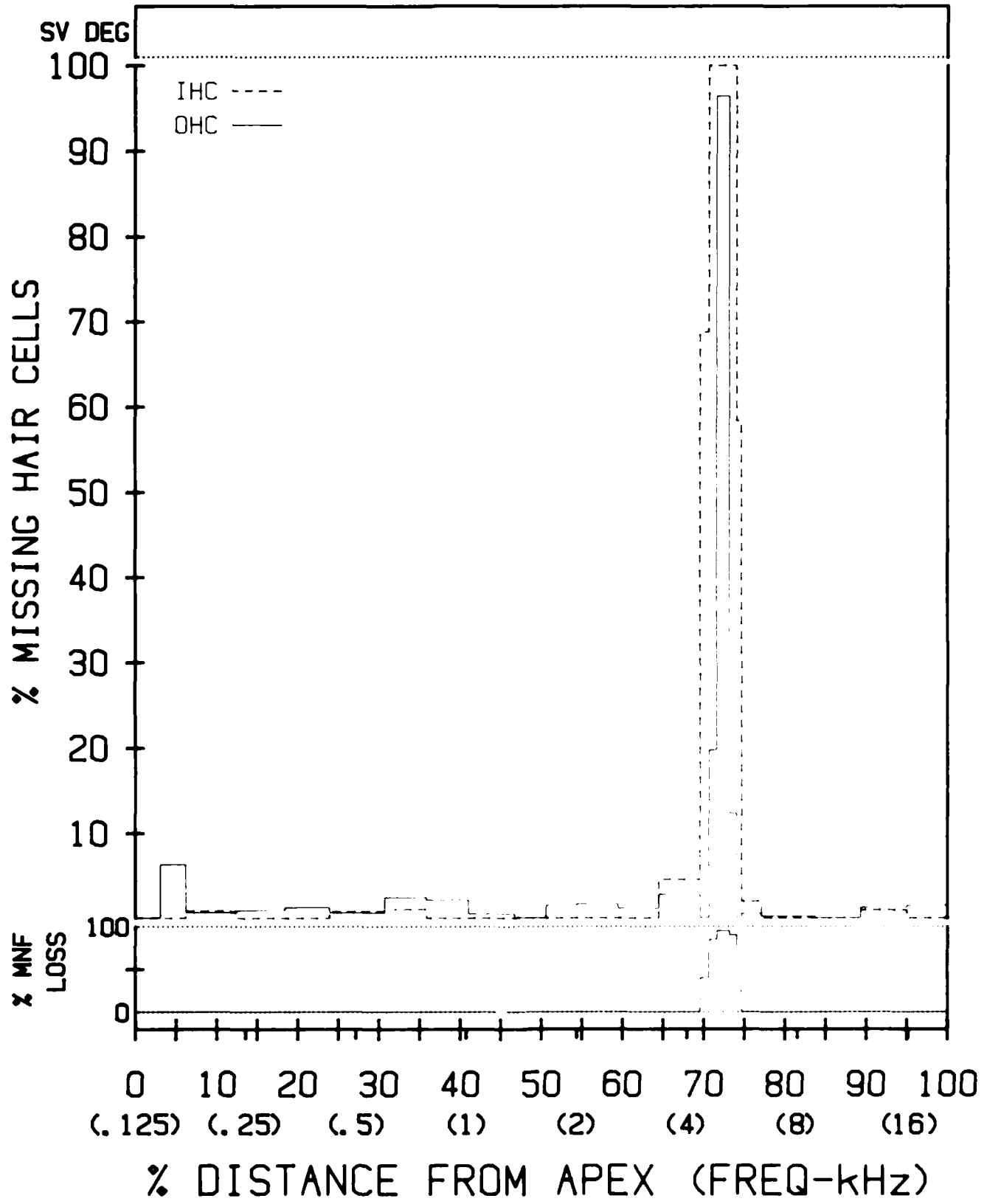

Figure 2: Cytocochleogram (713L) from sham-exposed chinchilla with a one-month recovery which had the largest OC lesion (0.93-mm combined HFL) in the group of shams. Note there is loss of MNFs associated with the HFL.

Cochlear damage in microwave-exposed chinchillas:

Acute Cochleas - Of the eight cochleas in this group, five (63%) sustained losses of OHCs in the 15-35% region (Table 6) which were more than three SDs larger than the average for controls. Two cochleas had a total of six IHC, OHC and combined HFLs which were located between 67.7% and 87.2% (Table 7). The IHC HFLs and the largest combined HFLs had an associated loss of MNFs (Table 7). In these lesioned cochleas, the summed size of the HFLs was 2.43 mm and 1.51 mm.

TABLE 6: COCHLEAR DAMAGE IN ACUTE MICROWAVE CHINCHILLAS (N=4)

	% MISSING HAIR CELLS		# MISSING PILLARS		SV DEG	MNF LOSS
EAR #	IHC (0-100%) (15-35%) (60-90%)	OHC	IP	OP	(Y/N)	(Y/N)
690R	7.4 0.3 22.6	8.6 6.1 20.5	60	84	N	Y
690L	10.1 0.3 32.4	12.9 8.6 33.3	29	99	N	Y
691R	0.6 0.2 0.9	4.9 16.0 0.4	0	0	N	N
691L	0.6 1.3 0.0	4.3 8.6 0.6	1	2	Y	N
695R	0.1 0.0 0.0	2.8 3.0 0.1	0	0	Y	N
695L	0.1 0.0 0.4	0.7 1.1 0.2	0	0	N	N
702R	1.8 0.8 0.2	1.1 1.0 0.2	0	0	N	N
702L	1.3 0.7 0.2	0.7 0.4 0.3	0	0	N	N

The cytocochleogram from the specimen with the most severe OC damage is shown in Figure 3. Two other cochleas had regions of SV degeneration: one had a 1.84-mm lesion from 1.2% to 8.5% and one had a 1.12-mm lesion from 66.6% to 72.4% (Table 8).

In summary, cochlear damage was found in all four acute microwave chinchillas. All animals had sizable differences between the pattern of damage in their right and left ears. However, there was no relation between the amount of damage in a particular ear and its orientation with respect to the microwave source.

TABLE 7: SIZE AND TYPE OF HFLs IN ACUTE MICROWAVE CHINCHILLAS WITH LESIONS

EAR #	HFL TYPE	EXTENT (mm)	% LOCATION	% MNF LOSS
690L	IHC	0.44	70.8-73.2	10
	COMBINED	1.99	75.1-85.7	0-75
690R	OHC	0.04	67.7-68.0	---
	COMBINED	0.04	71.8-72.0	---
	COMBINED	1.33	75.2-82.4	5-80
	COMBINED	0.10	86.6-87.2	---

TABLE 8: DEGENERATION OF STRIA VASCULARIS IN ACUTE MICROWAVE CHINCHILLAS WITH LESIONS

EAR #	EXTENT (mm)	% LOCATION
691L	1.84	1.2-8.5
695R	1.12*	66.6-72.4

*Surface of stria vascularis was bubbly and looked as if it was going to degenerate.

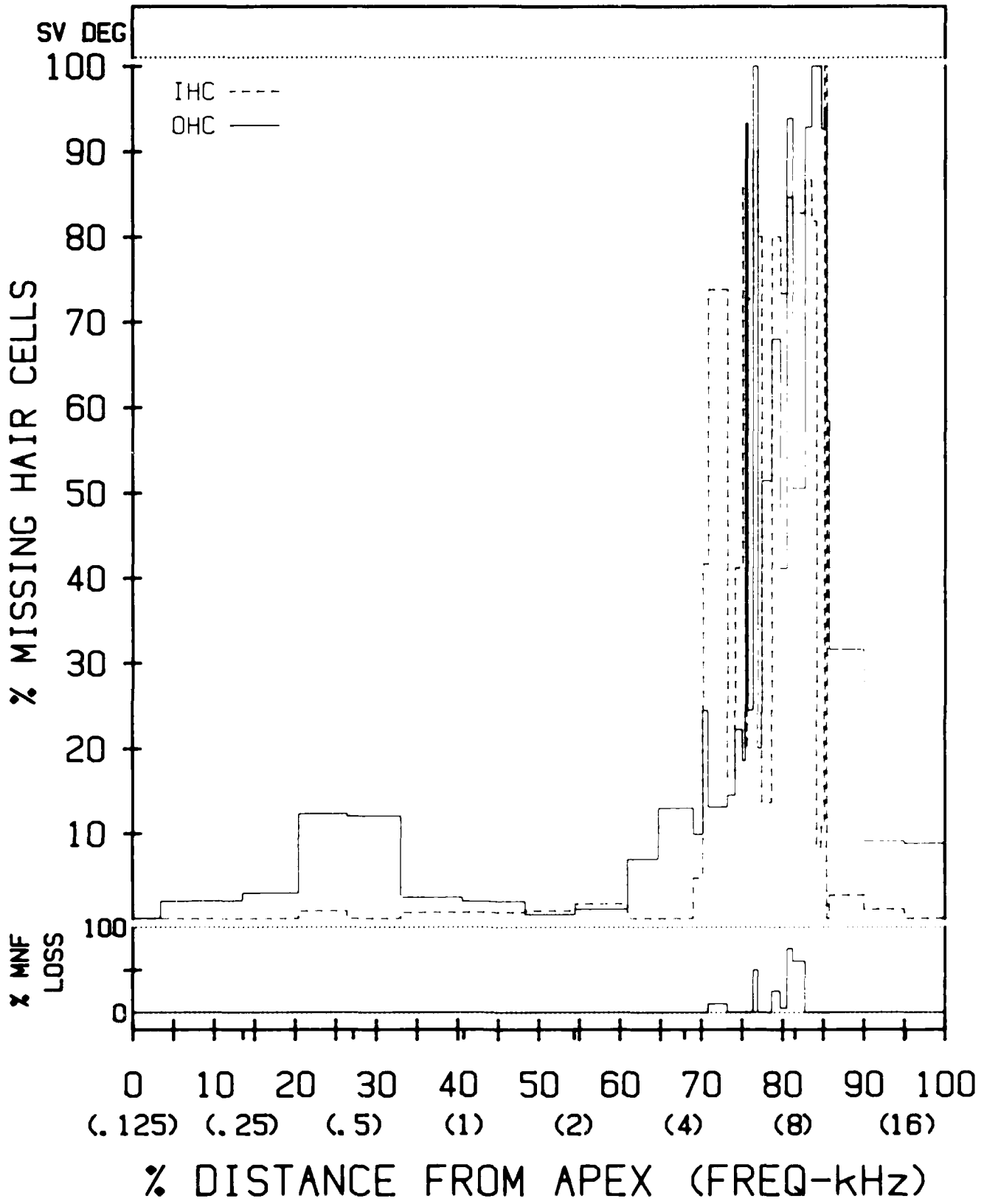

Figure 3: Cytocochleogram (690L) from microwave-exposed chinchilla with less than 24 hours of recovery which sustained significant loss of OHCs in the low-frequency region and had a 0.44-mm IHC HFL and a 1.99-mm combined HFL. Both HFLs had an associated loss of MNFs.

Chronic Cochleas - Twenty-one of 22 cochleas were evaluated quantitatively. A portion of one cochlea (711L) was lost during processing so quantitative data could not be obtained. One specimen had IHC loss and five had OHC losses in the 15-35% region (Table 9) which were more than three SDs larger than the average for controls. Eight cochleas had a total of 23 IHC HFLs, OHC HFLs or combined HFLs located between 64.2% and 83.8% (Table 10). There was a loss of MNFs associated with 74% of the HFLs (Table 10). For the individual cochleas with HFLs, the summed size of the lesioned area ranged from 0.03 mm to 1.51 mm and averaged 0.57 mm.

Ten cochleas each had a region of SV degeneration located between 54.0% and 78.0% which ranged in extent from 0.40 mm to 1.82 mm and averaged 1.26 mm (Table 11). The cytocochleogram from an ear which had lesions in the OC, MNFs and SV is shown in Figure 4. Figure 5 illustrates the cytocochleogram from the ear with the worst SV lesion.

In summary, cochlear damage was found in nine of 11 chronic microwave animals. Six animals had sizable differences between damage in their right and left ears; three animals had symmetrical lesions; one animal had no lesions in either ear; and the animal which had only one ear available for analysis had no lesions in that ear.

In summary, 13 of 14 chinchillas (93%) exposed to microwaves had some type of damage in at least one of their cochleas. One microwave animal had no lesions in either ear and the animal in which only the right ear was available for analysis had no lesions in this cochlea. By combining the data from the acute and chronic microwave cochleas (N=29), the following statistics were obtained: incidence of damage in the 15-35% region - 38% (11/29); incidence of HFLs - 34% (10/29); averaged summed size of HFLs - 0.85 mm; type of HFL (N=29) - 7% OHC HFLs, 66% IHC HFLs, 27% combined HFLs; incidence of SV degeneration - 41% (12/29); average summed size of SV lesion - 1.29 mm. Nine of the 13 (69%) microwave animals which had cochlear damage had asymmetrical lesions in their right and left ears.

TABLE 9: COCHLEAR DAMAGE IN CHRONIC MICROWAVE CHINCHILLAS (N=11)

EAR #	% MISSING HAIR CELLS IHC (0-100%) (15-35%) (60-90%)	OHC	# MISSING PILLARS IP	OP	SV DEG (Y/N)	MNF LOSS (Y/N)
703R	0.3	3.0	0	2	Y	N
	0.0	7.4				
	0.7	1.4				
703L	0.5	2.9	0	3	Y	N
	0.4	7.6				
	0.6	0.4				
704R	0.9	1.0	0	1	Y	N
	1.4	1.3				
	0.8	1.0				
704L	0.9	1.4	1	2	Y	N
	1.0	0.7				
	0.4	1.3				
706R	0.3	0.8	0	1	Y	N
	0.2	1.0				
	0.2	0.3				
706L	0.6	0.8	0	2	Y	N
	2.6	0.6				
	0.2	0.6				
707R	5.2	3.2	11	7	Y	Y
	0.3	2.4				
	16.5	5.8				
707L	6.1	3.7	17	10	Y	Y
	0.0	0.8				
	20.0	8.7				
711R	0.2	1.2	0	0	N	N
	0.0	0.5				
	0.2	0.3				
711L	No quantitative data; part of OC lost during processing.					
712R	0.4	1.2	3	1	Y	N
	0.0	1.2				
	0.3	1.9				
712L	0.6	1.1	1	1	N	N
	0.0	0.9				
	0.2	1.1				

TABLE 9 (CONT): COCHLEAR DAMAGE IN CHRONIC MICROWAVE CHINCHILLAS

EAR #	% MISSING HAIR CELLS IHC (0-100%) (15-35%) (60-90%)	% MISSING HAIR CELLS OHC	# MISSING PILLARS IP	# MISSING PILLARS OP	SV DEG (Y/N)	MNF LOSS (Y/N)
715R	0.7	3.1	3	14	N	Y
	1.5	1.3				
	0.6	7.9				
715L	0.6	0.8	1	1	Y	N
	1.5	1.4				
	0.0	0.3				
717R	2.1	1.5	2	4	N	Y
	0.2	1.8				
	7.0	2.0				
717L	3.3	1.4	3	0	N	Y
	0.3	1.2				
	10.1	1.5				
718R	0.2	0.5	0	1	N	N
	0.1	0.9				
	0.0	0.5				
718L	0.3	0.7	0	0	N	N
	0.5	1.7				
	0.0	0.1				
719R	0.4	2.8	1	3	N	N
	0.0	7.3				
	0.8	0.6				
719L	1.0	3.6	7	4	N	Y
	0.3	12.0				
	3.3	1.0				
721R	2.5	1.1	5	4	N	Y
	0.0	0.9				
	7.4	1.0				
721L	0.5	1.0	0	1	N	N
	1.5	1.5				
	0.5	0.4				

TABLE 10: SIZE AND TYPE OF HFLs IN CHRONIC MICROWAVE CHINCHILLAS WITH LESIONS

EAR #	HFL TYPE	EXTENT (mm)	% LOCATION	% MNF LOSS
704R	IHC	0.03	64.2-64.4	---
707R	COMBINED	0.17	70.5-71.4	5
	IHC	0.06	71.7-72.0	70
	IHC	0.23	72.5-73.7	5
	COMBINED	0.04	75.8-76.0	5
	IHC	0.39	77.1-79.1	15
	IHC	0.22	81.4-82.5	5-20
707L	COMBINED	0.95	69.3-74.2	0-30
	IHC	0.07	75.3-75.7	---
	IHC	0.12	78.0-78.6	---
	IHC	0.20	80.8-81.8	20
	IHC	0.04	82.0-82.2	10
	IHC	0.08	82.7-83.1	15
	IHC	0.05	83.5-83.7	---
715R	OHC	0.38	70.4-72.6	5
717R	IHC	0.29	79.7-81.2	90
717L	IHC	0.62	78.3-81.7	5-92
719L	COMBINED	0.02	70.2-70.3	---
	IHC	0.11	79.6-80.1	50
721R	IHC	0.16	75.2-76.0	75
	IHC	0.05	81.2-81.5	90
	IHC	0.05	81.7-81.9	---
	IHC	0.19	82.8-83.8	40

TABLE 11: DEGENERATION OF STRIA VASCULARIS IN CHRONIC MICROWAVE CHINCHILLAS WITH LESIONS

EAR #	EXTENT (mm)	% LOCATION
703R	1.52	60.7-67.2
703L	1.82	58.2-66.0
704R	1.55	58.9-65.4
704L	1.40	64.5-70.2
706R	0.93	72.0-76.8
706L	1.29	72.4-78.0
707R	0.40	65.6-67.1
707L	0.73	61.8-64.6
712R	1.38	59.3-64.9
715L	1.60	54.0-61.1

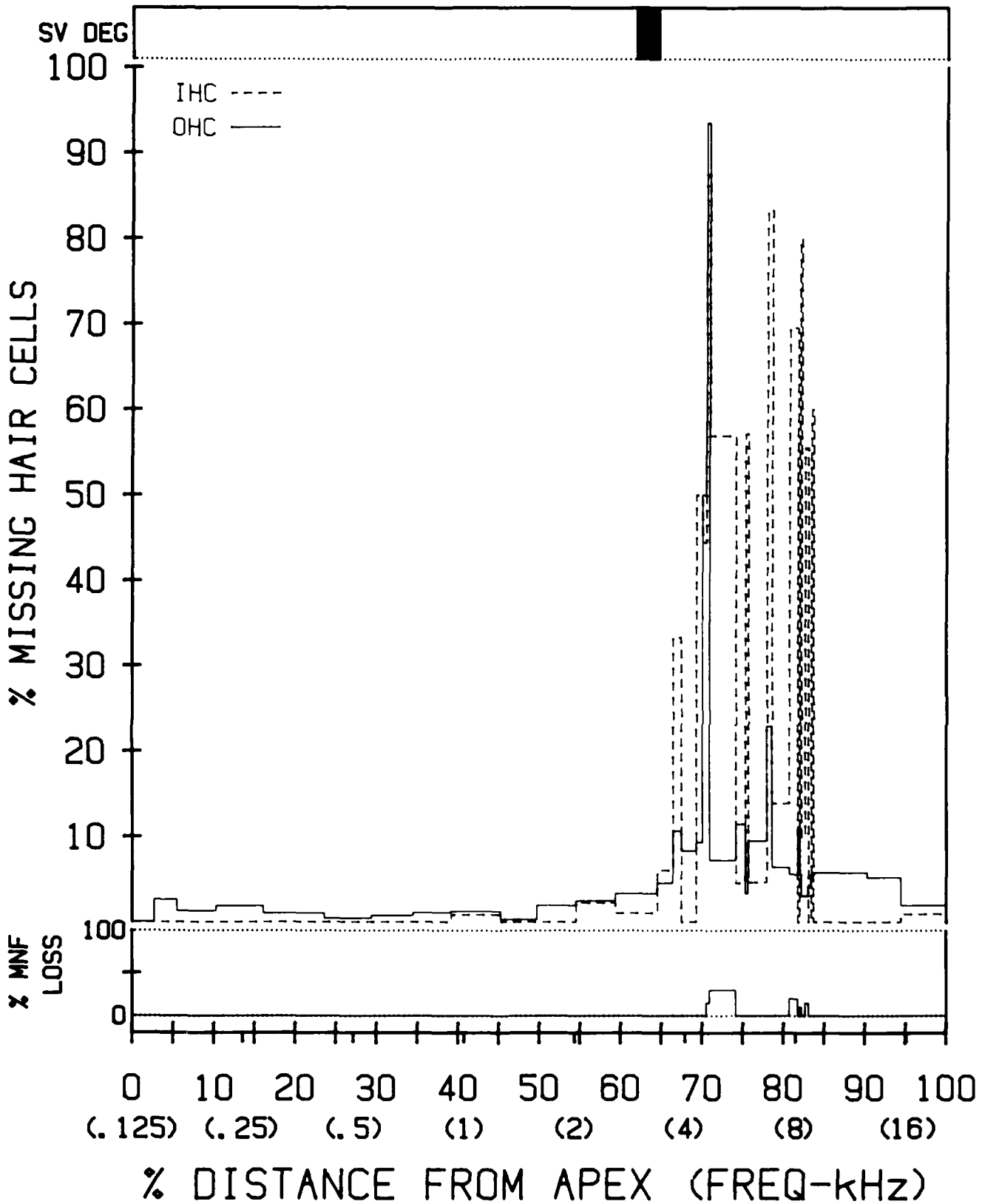

Figure 4: Cytocochleogram (707L) from microwave-exposed chinchilla with one-month recovery which had one 0.95-mm combined HFL with associated MNF degeneration and six IHC HFLs which ranged in extent from 0.04-0.20 mm. Two of the IHC HFLs had an associated loss of MNFs. This cochlea also had a 0.73-mm region of SV degeneration.

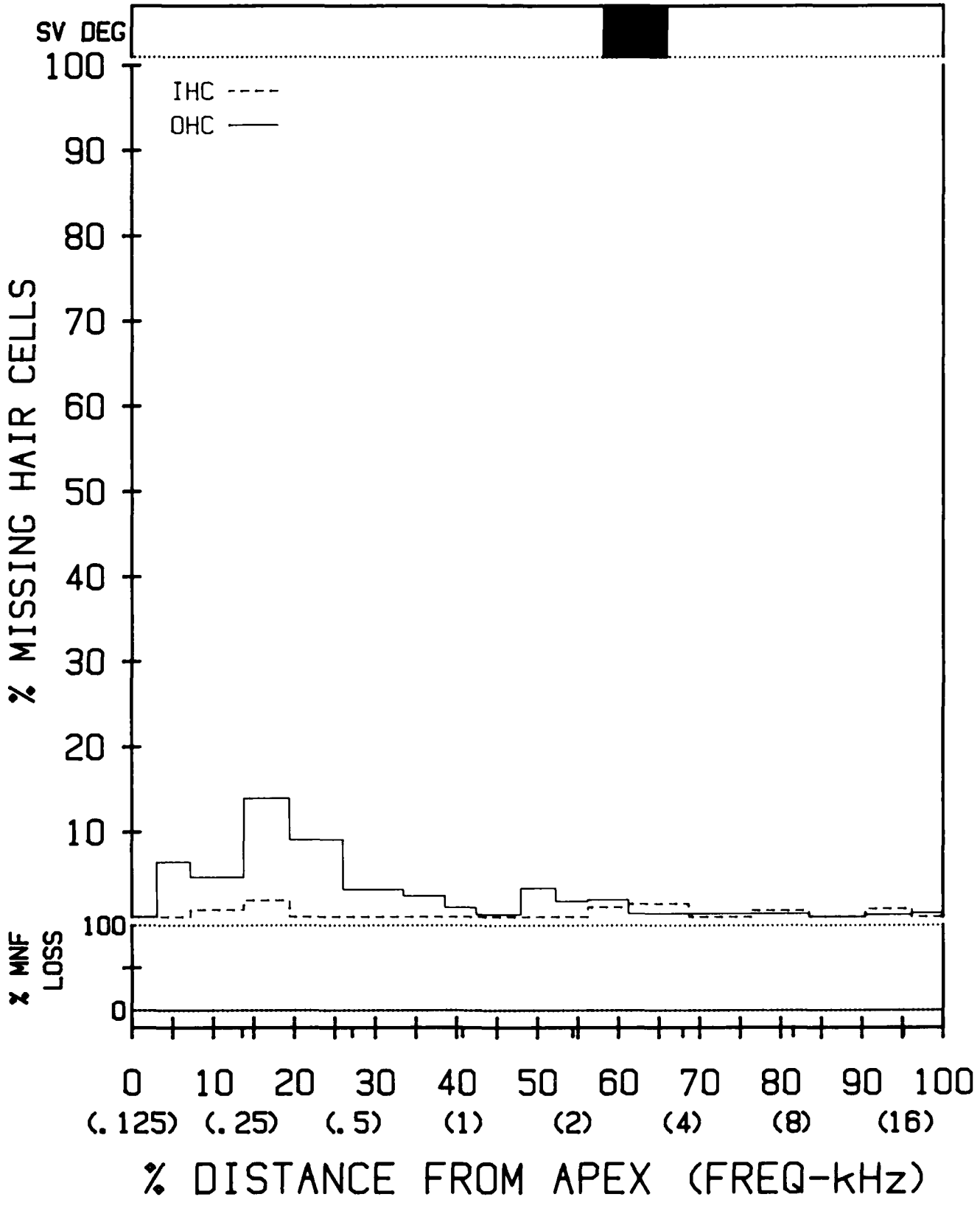

Figure 5: Cytocochleogram (703L) from microwave-exposed chinchilla with one-month recovery which had largest SV lesion. There was also elevated loss of OHCs in the low-frequency region.

DISCUSSION AND SUMMARY

Because of the similarities in the sham-exposed (N=12) and microwave-exposed (N=29) ears with respect to the incidence, pattern and magnitude of cochlear damage, their data are combined here and compared to those from cochleas damaged by excessive exposure to either low-frequency (i.e. 0.5 kHz) or high-frequency (i.e. 4 kHz) noise.

Cochlear damage in microwave study:

In the present study, 44% of the cochleas (18 ears - 7 sham and 11 microwave) sustained damage in the low-frequency region (15-35% distance from apex). In 17 of the 18 ears, the damage consisted of loss of OHCs scattered over a broad area of the OC. These losses ranged from 2.4-16.0% and averaged 6.7%. In the eighteenth ear, the damage consisted of 2.6% loss of IHCs.

In the high-frequency region, 39% of the cochleas (16 ears - 6 shams and 10 microwave) sustained damage. The damage consisted of narrow lesions in which there was severe loss of sensory cells, i.e. HFLs. In these 16 cochleas, there was a total of 41 HFLs which were located between 58% and 87.2%. The percentages of the different HFL types were as follows: 68% - IHC HFLs; 27% - combined HFLs; 5% - OHC HFLs. The summed size of the lesioned area in the damaged cochleas ranged from 0.03-2.43 mm and averaged 0.77 mm. Associated with 73% of the HFLs was a noticeable degeneration of some of the corresponding MNFs.

Degeneration of the SV was found in 34% of the cochleas (14 ears - 2 shams and 12 microwave). Twelve of the cochleas had the lesions located between 54% and 78% which ranged in size from 0.40-1.82 mm and averaged 1.20 mm. The other two cochleas had SV lesions which averaged 2.11 mm in extent and were located between 1.2% and 11.8%.

Cochlear damage from noise exposure:

Exposure of chinchillas for 2-36 days to an octave band of noise (OBN) with a center frequency of 0.5 kHz and a sound pressure level (SPL) of 95 dB resulted in OHC loss in the low-frequency region ranging from 9-29% (Bohne and Clark, 1982). The same exposure also damaged the high-frequency region of the OC in 64% of the cochleas (35/55). The 35 lesioned cochleas had a total of 70 HFLs located between 50-98% and distributed in the following percentages: 54% combined HFLs; 29% OHC HFLs; and 17% IHC HFLs. Degeneration of a portion of the SV was fcund in 15% of the cochleas (8/55). The lesions ranged from 1.13-2.58 mm in extent, averaged 1.69 mm and were located between 58.8-86.6% (Bohne and Gruner, unpublished data).

Chinchillas which were exposed for 2-36 days to a 4-kHz OBN at 80 or 86 dB SPL sustained damage in the high-frequency region of the OC (Bohne et al, 1987). One or more HFLs were found in 68% of the cochleas (21/31). The 21 lesioned cochleas had a

total of 47 HFLs located between 63-100% and distributed in the following percentages: 45% combined HFLs; 38% OHC HFLs; and 17% IHC HFLs. None of these cochleas had any regions of SV degeneration (Bohne and Gruner, unpublished data).

Comparison of microwave data to noise data:

Although the inner ear damage found in the sham- and microwave-exposed chinchillas is qualitatively similar to that resulting from excessive exposure to noise, there are some important differences. First, many of the animals in the microwave study had asymmetrical lesions. Most chinchillas which receive free-field exposures to noise for more than 24 hours have symmetrical lesions in their right and left cochleas (Bohne et al, 1986). Secondly, a high percentage of IHC HFLs was found in the ears in this study whereas the percentage of IHC HFLs was much lower in ears which were damaged by exposure to low- or high-frequency noise. Thirdly, basal-turn damage was confined to a relatively narrow portion of the basilar membrane in the microwave ears even though the equivalent SPL of the microwave exposure was in the range of 170 dB at 60 kHz. Chinchillas exposed to low- or high-frequency noise at SPLs of 120 dB or greater have severe damage throughout the basal portions of their cochleas (Bohne and Bozzay, unpublished data).

Based on the quantitative and statistical differences between the ears in the present study and those damaged by noise, it is highly unlikely that damage found in the microwave ears was the result of exposure to environmental noise. Review of the data from the control chinchillas indicates that it is also unlikely that the damage in the microwave ears was pre-existing. On the other hand, since the lesions in the sham- and microwave-exposed cochleas are so similar, the damage cannot be attributed solely to exposure to microwaves. It is concluded that some unidentified ototraumatic agent at the WRAIR Microwave Laboratory was responsible for the cochlear damage seen in both the sham- and microwave-exposed animals.

RECOMMENDATIONS

Because of the high incidence of damage in the sham-exposed cochleas, we were not able to determine if exposure to microwaves represents a potential hazard to the inner ear. In order to address this question, additional studies must be conducted. In an attempt to avoid the problems encountered in the present study, it is suggested that the following points be incorporated into the next experimental design:

1. The experimental animals should be exposed to microwaves and their ears prepared for histological examination in the same city. Use of this strategy will eliminate shipping stress, transportation noise, dietary changes, etc. as potential additive or multiplicative factors in generating inner ear damage.

2. The experimental animals should not be housed in the same building as that which houses microwave or other electromagnetic equipment unless it is absolutely certain that there is no leakage of electromagnetic waves from the equipment.

3. Some of the sham- and microwave-exposed animals should have one of their external and/or middle ears blocked (e.g. earplug or ossicular disarticulation) so that there will be a 50-60-dB attenuation of airborne sound entering the inner ear. This procedure may enable researchers to determine whether or not the noise from the microwave generating equipment was a complicating factor in the present study.

4. The orientation of the animals with respect to the microwave source should be precisely maintained throughout the sham and microwave exposures. This procedure will enable researchers to minimize some of the interanimal variations in the exposure which occurred as a result of differences in orientation.

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