AD_____

GRANT NUMBER DAMD17-96-1-6330

TITLE: Sex Difference in Susceptibility and Resistance to Noise-Induced Hearing Loss in Chinchillas

PRINCIPAL INVESTIGATOR: Sandra L. McFadden, Ph.D.

CONTRACTING ORGANIZATION: The State University of New York
at Buffalo
Amherst, New York 14228-2567

REPORT DATE: October 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Annual (23 Sep 97 - 22 Sep 98)

4. TITLE AND SUBTITLE
Sex Differences in Susceptibility and Resistance to Noise-Induced Hearing Loss in Chinchillas

5. FUNDING NUMBERS
DAMD17-96-1-6330

6. AUTHOR(S)
Sandra L. McFadden, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The State University of New York at Buffalo
Amherst, New York 14228-2567

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Considerable evidence points to gender differences in susceptibility to noise-induced hearing loss (NIHL), a major occupational hazard for military personnel. However, the precise nature of these differences and how they apply to the types of noises found in military settings is unknown. Five experiments were conducted to explore these issues. Experiments conducted during Year 1 showed that chinchillas exhibit sex differences in basic auditory sensitivity and susceptibility to temporary and permanent hearing loss caused by exposure to simulated M16 rifle fire. Sound conditioning experiments completed during Year 2 show that pre-exposing animals to a low-frequency (0.5 kHz octave band) noise at 90-95 dB SPL for 5 days provides significant protection from subsequent exposure to M16 rifle fire, whereas exposing animals to simulated Blackhawk helicopter noise at 112 dB SPL for 5 days has little protective effect. The results of the five experiments point to sex differences in the response of the cochlea to noise and the ability to develop resistance to noise through prophylactic conditioning exposures, which could have important implications for military assignments and hearing conservation programs.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Defense Women's Health Research Program Acoustic trauma: Gender differences: Hearing impairment: Noise-induced hearing loss: Ovarian cycle: Toughening	58
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

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

SLK In conducting research using animals, the investigator(s) 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 Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Sandra L McFadden 10-8-98

PI - Signature Date

TABLE OF CONTENTS

1. Introduction

Noise-induced hearing loss (NIHL) is a major occupational hazard for military personnel (Dancer and Franke, 1986; Henselman, Henderson, Subramaniam, and Sallustio, 1994; Henselman, Henderson, Shadoan, Subramaniam, Saunders, and Ohlin, 1995). Soldiers are exposed to continuous noise inside armored vehicles, planes, helicopters and ships, and to impulse noise from weapon fire and explosions. Exposure to these noises can produce extensive damage to the cochlea, resulting in permanent and debilitating hearing loss. Continuous noise can damage the cochlea indirectly, by triggering metabolic and biochemical changes that lead to cellular dysfunction and sensory cell death. Impulse noises can damage the cochlea both indirectly and directly, by producing mechanical damage such as ripping the organ of Corti away from the basilar membrane (Dancer and Frank, 1986; Henderson, Hamernik, and Sitler, 1974; Henderson, Spongr, Subramaniam, and Campo, 1994; Spondlin, 1976). NIHL affects the health and safety of military personnel, and costs the government millions of dollars in personal compensation and medical expenses every year. In the 20-year period between 1976 and 1996, the US government spent over $3.5 billion on hearing loss as a primary disability (Sherris, personal communication).

A recognition of the serious consequences of NIHL prompted the U.S. military services to develop hearing conservation programs (HCPs), beginning with the U.S. Air Force in 1948 (Henselman et al., 1995). Military HCPs have served to increase awareness of the damaging effects of noise, and have mandated the use of personal protection devices (PPDs) such as sound-attenuating ear plugs or earmuffs in high-noise situations. Despite these efforts, NIHL remains a serious problem for soldiers who are exposed to loud noises in training and combat situations in which PPDs are either unavailable, impractical or dangerous to use, improperly fitted or worn, or inadequately designed to protect the ear from damage (Dancer et al., 1998). Despite the best efforts of the military to prevent NIHL, compensation costs for hearing loss have risen steadily over the years. In 1976, the cost of hearing loss as a primary disability for all veterans was approximately $70 million. In 1996, this figure had grown to more than $254 million (Sherris, personal communication).

As women become more fully integrated into a variety of military occupational specialties, many will be placed at risk for developing NIHL. It is critical, therefore, that we understand the specific relationship between noise exposure and hearing loss in women, so that appropriate measures for preventing NIHL can be developed and implemented. Previous studies of gender differences in susceptibility to NIHL have focused on temporary threshold shifts (TTS) caused by continuous tones and noise. These studies have shown that males exhibit more TTS than females from low-frequency exposures (below 2 kHz), whereas females exhibit more TTS than males from high-frequency exposures (above 2 kHz). The more important issue of gender differences in susceptibility to permanent threshold shift (PTS) has not been amenable to study. Until now, the only data addressing sex/gender differences in susceptibility to PTS have come from retrospective studies of hearing loss in workers exposed to noise in industrial settings (Berger, Royster, and Thomas, 1978; Gallo and Glorig, 1964). Under these conditions, which typically involve exposure to low-frequency continuous noises, males tend to develop much more hearing loss than females. Both Berger et al. (1964) and Gallo and Glorig (1964) found approximately 20 dB more PTS in males than in females after nine years of industrial noise

exposure. There are no comparable studies of sex/gender differences in PTS produced by exposures to high-level impulse noises. A finding of gender differences in susceptibility to NIHL from impulse noise could have important implications for military assignments and hearing conservation programs.

The current project has two primary goals. The first goal is to determine if sex differences exist in basic auditory sensitivity and in the response of the cochlea to high-level impact noise, using an animal model in which sex differences are not confounded by noise exposure history, diet, recreational activities, or other extraneous factors that can influence hearing loss. The second major goal is to explore ways of reducing or preventing NIHL. Experiments conducted during the first two years of the project have established that (a) chinchillas show small but consistent sex differences in basic auditory sensitivity that parallel those observed in humans, and (b) there is a fundamental sex difference in the response of the chinchilla cochlea to high-level impact noise. Female chinchillas tend to have lower high-frequency thresholds, and higher low-frequency thresholds than males. After exposure to simulated M16 rifle fire, female chinchillas developed approximately 10 dB more high-frequency hearing loss, but 5 dB less low-frequency hearing loss than males. These findings suggest that gender differences in humans as well as chinchillas are related to inherent anatomical and physiological differences, rather than to systematic differences in noise exposure history.

Current experiments are exploring ways of reducing the harmful effects of noise through prophylactic "conditioning" noise exposures and novel pharmacological approaches. Experiments conducted during Year 1 began to explore the feasibility of protecting the ear from impulse noise through sound conditioning, using either helicopter noise or 0.5 kHz octave band noise (OBN). The initial results from experiments using noise that simulated UH-60 Blackhawk helicopter noise showed little benefit in terms of protection from subsequent exposure to M16 rifle fire. Pre-exposing animals to the helicopter noise at 90 dB SPL for 5 days produced 5 dB or less protection from M16 rifle fire. A 10-day series of exposures to the helicopter noise at 112 dB SPL resulted in no protection for males, and potentiation of damage from subsequent M16 rifle fire for females, by approximately 10-15 dB. In contrast, females conditioned with 0.5 kHz OBN at 90-95 dB SPL showed substantial protection (up to 15 dB SPL) from M16 rifle fire. With regard to sex differences, females conditioned with 0.5 kHz OBN showed up to 12 dB more resistance to PTS than males. In Year 2, we have expanded our study of the protective effects of sound conditioning. The 0.5 kHz OBN conditioning experiment was completed, and an experiment examining the effects of shortening the duration of the 112 dB SPL helicopter noise exposure to 5 days on hearing loss and hair cell loss was conducted. In addition, we have begun to explore the use of pharmacological agents to prevent PTS. The rationale for these studies of cochlear protection, and the initial intriguing results, are described in appropriate sections below.

This report describes the advances made toward the two major goals of the project during the second year of funding. Specifically, the results describe (a) sex differences in noise-induced cochlear pathology (i.e., loss of inner and outer hair cells); (b) the ability to benefit from low-frequency conditioning exposures; (c) the effects of UH-60 helicopter noise on hearing and protection from NIHL; and (d) the ability to benefit from pharmacological intervention with R-phenylisopropyladenosine (R-PIA) and 17-β-estradiol (E_2). The findings from the chinchilla

offer insights into gender differences in susceptibility to impact/impulse noise, and the feasibility of using pharmacological approaches to prevent or reduce NIHL.

2. Body

2.1. Experimental Methods

All procedures described here were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to NIH guidelines for the humane treatment of laboratory animals.

2.1.1. Subjects and experimental groups

A total of 32 chinchillas (*Chinchilla langier*) were used for experiments during Year 2 of the project. Animals were between 1 and 3 years of age, and weighed between 430 and 725 g. The chinchilla was used for these studies because it (a) is relatively immune to middle ear infections and diseases that affect hearing (Clark, 1984); (b) has a relatively long life span (12-20 years) with minor age-related cochlear pathology and hearing loss prior to 8-10 years of age (Bohne, Gruner, and Harding, 1990; McFadden, Campo, Quaranta, and Henderson, 1997); and (c) reacts predictably to anesthesia and tolerates surgery well. Most importantly, the chinchilla has a range of hearing that is more similar to that of humans than most other laboratory animals, particularly in the low frequencies (Miller, 1970; Heffner and Heffner, 1991), which enhances its suitability as a model for studying NIHL (McFadden, Campo, Ding, and Quaranta, 1998). With regard to size, Clark (1984) states that female chinchillas tend to be larger than males. In a small group of our chinchillas (8 female, 8 male) for which reliable weights were available, weight differences were minor, but favored females. The average weight of females was 572.2 g (SD= 73.7), versus 563.9 g (SD=70.0) for males.

The experimental groups for Year 2 are summarized in Table 1. The third column of the table, noise configuration, refers to whether one or two acoustic drivers were used for the impulse noise exposure (see Section 2.1.5).

TABLE 1: Groups and Number of Ears Tested During Year 2.

GP	PRE-TREATMENT	150 dB pSPL NOISE CONFIGURATION	# EARS TESTED F	M
1.	0.5 kHz OBN, 90-95 dB SPL, 6 h/d, 5d	#1 (2 Drivers)	5	5
2.	Helicopter noise, 112 dB, 1.5 h/d, 5 d	#1 (2 Drivers)	5	6
3.	NONE	#2 (1 Driver)	2	5
4.	R-PIA	#2 (1 Driver)	2	4
5.	17-β-Estradiol or Vehicle	#2 (1 Driver)	5	4
		TOTAL	19	24

2.1.2. Surgical preparation for evoked potential recording

All animals were prepared for evoked potential (EVP) recording by surgically implanting tungsten electrodes into the right and/or left inferior colliculus (IC). Each animal was deeply anesthetized with an intramuscular injection of ketamine hydrochloride (60 mg/kg) and acepromazine (0.56 mg/kg). A midline incision was made through the skin overlying the skull, and a small hole was made in the dorsal cranium overlying the IC. A tungsten electrode, approximately 2.5 cm long, was inserted through the hole and advanced through the IC to a depth that produced a clear, large-amplitude response to a click stimulus at approximately 80 dB SPL. The electrode was cemented to the skull with cyanoacrylic adhesive and dental cement. A short tungsten electrode, approximately 1.25 cm long, was implanted in the rostral cranium to serve as the common lead for IC-EVP recording.

The electrodes were made by removing the insulation from each end of a Teflon-coated tungsten wire (0.216 mm diameter bare; 0.279 mm coated) and soldering a gold-plated male connector pin onto one end. The relatively large, low-impedance (typically less than 100 kΩ) recording electrode picks up electrical activity from a very wide region of the IC. Regardless of the exact position of the electrode tip, responses to both low and high frequencies can be recorded, and thresholds are independent of electrode depth and mediolateral/rostrocaudal position. Because the electrodes remain fixed in position, variability associated with changes in electrode placement between tests is eliminated. In addition, the signal-to-noise ratio is much better with implanted electrodes than with more conventional scalp electrodes, resulting in threshold estimates that are very close to behavioral thresholds measured in the same animals (Henderson et al., 1983), and about 15-20 dB lower than threshold estimates obtained using subcutaneous electrodes in the same animals (Murphy and Themann, 1995).

Following surgery, the animals recovered in a quiet animal colony for at least one week prior to testing.

2.1.3. Measures of auditory function

The auditory sensitivity of each animal was assessed by measuring IC-EVPs. Cubic ($2f_1$-f_2) distortion product otoacoustic emissions (CDPs) were also obtained from some animals. However, due to equipment malfunction, we do not consider the CDP data obtained from animals in Year 2 to be reliable. Consequently, this report will focus exclusively on the results of IC-EVP testing. We do not consider this to be a limitation, however, as recent studies have shown that IC-EVPs are selectively sensitive to pathology of the outer hair cells (McFadden et al., 1998b), and provide essentially the same information as CDPs (McFadden and Campo, 1998).

All testing was conducted in a sound-attenuating booth (Industrial Acoustics Corp. 400) lined with sound-absorbing foam panels. The awake chinchilla was placed in a custom-designed tube (Snyder and Salvi, 1994) that held its head at a constant orientation within the calibrated sound field. Stimuli consisted of 10 ms tones (2 ms cosine rise/fall ramp, alternating phase) at octave intervals from 0.5 to 16 kHz, presented at a rate of 20/sec. Stimuli were generated digitally (93 kHz sampling rate) by a 16 bit D/A converter on a digital signal processing (DSP) board (TMS320C25) in a personal computer (PC), and routed through computer-controlled attenuators and impedance matching transformers to a loudspeaker (Realistic 401197) located on the side of

the test ear, at a distance of approximately 9 cm from the animal's pinna. The opposite (non-test) ear was plugged with a foam insert earplug, which provides approximately 20-40 dB attenuation (see Table 2) in addition to the 5-10 dB attenuation produced by the animal's head and body obstructing the propagation of sound to the opposite ear. Electrical activity from the IC electrode contralateral to the test ear was amplified (20,000 X), filtered (10-3000 Hz), and converted to digital signals (50 kHz sampling rate) by an A/D converter on a separate DSP board. Stimuli were presented in ascending order of frequency and intensity. Fifty or 100 trials were computer averaged at each stimulus level and the level was incremented in 5 dB steps. Stored waveforms were analyzed visually to determine thresholds. Threshold (dB SPL re: 20 μPa) was defined as the mid-point between the level at which there was a clear deflection in the waveform and the next lower level at which there was none. For example, if there was a clear response at 5 dB SPL and none at 10 dB SPL, the threshold was recorded as 7.5 dB SPL.

Table 2: Attenuation provided by a foam insert earplug (values are means of measurements obtained from 4 animals).

0.5 kHz	1 kHz	2 kHz	4 kHz	8 kHz	16 kHz
20.00	23.75	26.25	27.50	40.00	35.00

2.1.4. Conditioning noises and acoustic calibration

Noise stimuli used for "conditioning" exposures consisted of 0.5 kHz OBN at 90-95 dB SPL, 6 hr/day for 5 days, or helicopter noise at 112 dB SPL for 1.5 hr/day for 5 days. The 0.5 kHz conditioning noise was digitally generated, low-pass filtered (TDK HAF0030 active filter set at 20 kHz), manually attenuated (Hewlett Packard 350D), amplified (NAD 2200), and delivered to a compression driver (JBL 2446J) fitted to a bi-radial exponential horn (JBL 2360H). The driver/horn assembly was suspended from the ceiling of a sound booth (Acoustic Systems Model RE-132). The helicopter noise was digitized from a cassette recording made from the cabin of a U.S. Army UH-60 Blackhawk helicopter while the aircraft was cruising at a speed of 120 knots (Henselman, 1995). The digitized helicopter noise was routed from the PC to a graphic equalizer (Technics SH 8065). Output from the graphic equalizer was routed to two band-pass filters (Krohn-Hite 3550), one set for 10-2000 Hz, the other for 1000-10,000 Hz. Output from each band-pass filter was amplified by separate amplifiers (NAD 2200) and attenuated by separate attenuators (HP 350D). The low-frequency portion of the UH-60 helicopter noise was delivered to a woofer (Power Logic HT 615) housed in a wooden enclosure located in one corner of the sound booth, while the high-frequency portion was delivered to the compression driver and horn suspended from the ceiling of the booth.

For acoustic calibration of the conditioning noises, sound pressure levels (SPL re: 20 μPa) were measured with a calibrated Type I precision sound level meter (Larson-Davis 800B) and a 1/2" condenser microphone positioned at a height corresponding to the level of the ear canal of a standing chinchilla. SPL measurements were averaged across 5 positions within each cage (geometric center and each corner). Attenuator settings and cage positions were adjusted so that the average SPL in each cage was within 1 dB of the specified SPL. Figure 1 shows the

measured spectrum of the helicopter noise. Figure 2 shows the measured spectrum of the 0.5 kHz OBN.

Fig. 1. Spectrum of the simulated UH-60 Blackhawk helicopter noise (1/3 octave band measurements).

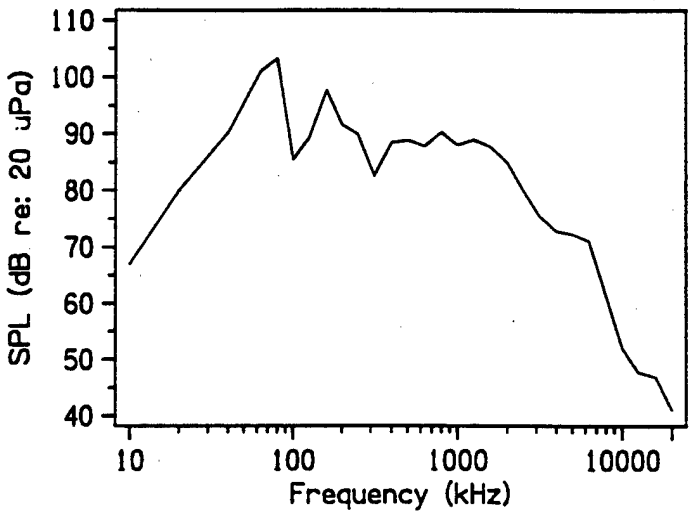
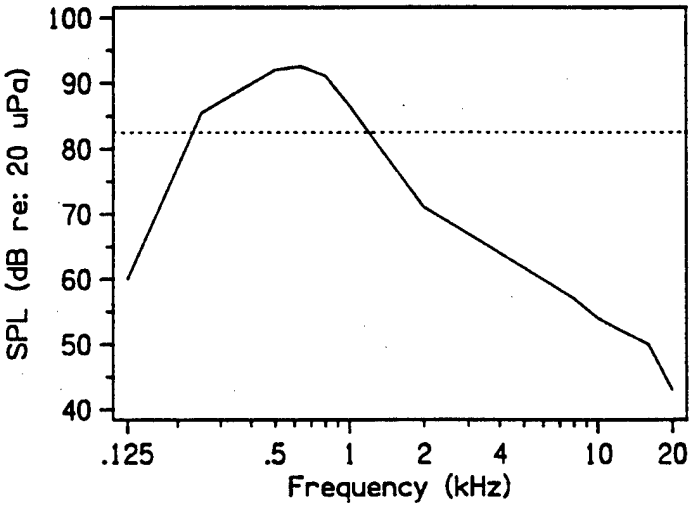


Fig. 2. Spectrum of the 0.5 kHz OBN conditioning noise (1/3 octave band measurements).



During exposure to conditioning noise, animals were housed in separate cages (approximately 27 cm X 21 cm X 22 cm) placed beneath the loudspeaker, and provided free access to food and water. Animals were rotated to different cages each day to minimize any effects of slight differences in SPL between cages.

2.1.5. Impulse noise and acoustic calibration

All animals described in this report were exposed to 150 dB peak SPL impulse noise. The impulse noise was a modified Friedlander wave (0.8 ms A-duration) with a time-amplitude profile simulating impulses created by 5.56 mm rounds fired from a U.S. Army M16A1 rifle (Danielson, Henderson, Gratton, Bianchi, and Salvi, 1991). The digital signal was routed to a D/A converter on a DSP board, attenuated (HP 350D), and amplified (NAD 2200). Electrical signals were converted to acoustic impulses by a compression driver (JBL 2446) coupled to a sound delivery tube (5 cm dia X 20 cm) whose end was cut at a 45° angle to broaden the range of the tube's resonance (Danielson et al., 1991). Two different methods of delivering the noise to the animal were used. In the first method ("noise configuration #1"), two separate acoustic drivers were used. The two drivers faced each other, with the sound delivery tubes separated by 10 cm. An animal was placed in a restraint tube in the 10 cm space between the opposing sound tubes. In the second method ("noise configuration #2"), a single driver was placed in front of the animal (0° azimuth), 10 cm from the

interaural plane. All other parameters of the noise exposure (described below) were the same for the two driver configurations.

Fifty pairs of impulses (100 total) were delivered simultaneously to both ears of an animal. Impulses in each pair were spaced 50 ms apart, and there was a 1000 ms interval between the onset of each pair (Henselman et al., 1994). The duration of the exposure was therefore less than one minute for each animal. For calibration of the impulse noise, a 1/8" microphone (Bruel and Kjaer Model 4138) was placed at the position that would be occupied by a restrained animal. The voltage corresponding to a 114 dB, 250 Hz tone produced by a pistonphone coupled to the microphone was determined, and used to calculate the desired voltage for a 150 dB peak SPL signal. The attenuation was adjusted to produce the desired voltage.

2.1.6. Test schedule for sound conditioning experiments

In experiments involving conditioning noise, IC-EVPs were recorded (a) prior to noise exposure in order to establish pre-exposure baselines, (b) during the exposure period in order to monitor TTS caused by the conditioning noise, (c) 5 days after conditioning to document recovery from TTS, (d) 15 min, 24 hr, and 5 days after high-level exposure (noise configuration #1) in order to monitor recovery from TTS, and (e) after 20-30 days recovery from high-level exposure in order to determine permanent loss of auditory sensitivity. Prior to exposure, each animal was tested three times, and the average of the three measurements was used as the stable baseline estimate of sensitivity. Threshold shifts were calculated by subtracting mean pre-exposure IC-EVP thresholds from post-exposure thresholds. After 20-30 days recovery from high-level exposure, IC-EVPs were measured on three separate occasions and averaged in order to calculate PTS at each frequency.

2.1.7. Procedures for pharmacological intervention experiments

2.1.7.1. R-phenylisopropyladenosine (R-PIA) pilot study

Animals (2 females, 5 males) were anesthetized with an intramuscular injection of ketamine (60 mg/kg) and acepromazine (0.5 mg/kg) and a small hole was made in each bulla to access the round window membrane. Approximately 30 µl of R-PIA (10^{-3} M) in physiological saline was dropped onto the right round window of each animal. An equal volume of saline was applied to the left round window in 3 animals, and nothing was done to the left ear of 4 animals. The holes in the bullae were sealed with dental cement and the skin incision was sutured closed. Baseline audiograms were measured after the R-PIA was applied but before the noise exposure. Two hours after application of R-PIA, the animals were exposed to impulse noise (noise configuration #2).

2.1.7.2. 17-β-estradiol pilot study

Animals were randomly assigned to an estradiol treatment group (2 females, 2 males) or a control group (3 females, 2 males). Animals in the treatment group received daily subcutaneous injections of 17-β-estradiol (50 µg in 0.2 ml olive oil vehicle) for 14 consecutive days. Animals in the control group received an equal volume (0.2 ml) of vehicle alone for 14 days. One day after impulse noise exposure (noise configuration #2), animals in the estradiol group received a subcutaneous injection of 0.5 ml estradiol in olive oil vehicle, and animals in the control group received 0.5 ml of vehicle alone. All animals were handled and treated identically during the experiment. IC-EVP thresholds were measured before treatment (2X), after 2, 4, and 7 days of

treatment, and at 15 min, 24 hr, 7 days, and 14 days after impulse noise exposure (noise configuration #2). Like pre-exposure thresholds, PTS measures represent the average of two threshold measurements, usually obtained on consecutive days.

2.1.8. Cochlear histology

Chinchillas were deeply anesthetized with sodium pentobarbital (Somlethal, 100 mg/kg i.p.) and decapitated. The cochleas were quickly removed and perfused through the oval window with a succinate dehydrogenase (SDH) staining solution consisting of 2.5 ml of 0.2 M sodium succinate, 2.5 ml of 0.2 M phosphate buffer (pH 7.6), and 5 ml of 0.1% tetranitro blue tetrazolium. Cochleas were incubated in the SDH staining solution for 45 min at 37 °C, post-fixed with 10% formalin, and stored in fixative for at least 24 hours. Stained cochleas were dissected from the apex to the base, mounted in sections in glycerin on microscope slides, coverslipped, and examined using light microscopy (400X magnification). The numbers of missing OHCs and IHCs were determined for successive segments of the organ of Corti. Individual cochleograms were constructed to show the percentage of hair cells missing as a function of distance from the apex of the cochlea. Percent hair cells missing was referenced to our lab standards based on average hair cell counts from 9 cochleas of young (<1 yr old), healthy chinchillas. Percent distance from the apex was converted to frequency using the frequency-place map of Greenwood (1990).

2.1.9. Data analyses

Data analyses were geared toward answering the following questions: (1) Do female and male chinchillas differ in their responses to conditioning noises? (2) Do female and male chinchillas derive equivalent benefit (if any) from conditioning exposures, in terms of protection from PTS caused by high-level exposure? (3) Are there sex differences in cochlear hair cell losses after noise exposure? (4) Does pre-treatment with R-PIA reduce NIHL? (5) Does estradiol treatment alter susceptibility to NIHL? Analyses of variance (ANOVAs) and Student t-tests were used to assess differences between means. The dependent variables were IC-EVP thresholds and threshold shifts at various times after noise exposure. Independent variables were Sex and Group (between-subjects factors), and Frequency and Time of Assessment (within-subjects factors). Significant main effects and interactions were analyzed further using t-tests. Within a group, changes as a function of time or frequency were assessed using paired t-tests. All statistical tests were evaluated using a 0.05 criterion of significance.

2.2. Results and Discussion

This report describes research conducted from September 23, 1997 to September 22, 1998 (Year 2) to complete the five experiments outlined for the first two years (Phase I) of the project. Experiments performed during Year 1 (1996-1997) focused on sex differences in: (1) auditory sensitivity prior to noise exposure, (2) susceptibility to hearing loss caused by high-level impulse noise exposure (noise configuration #1), and (3) the ability to benefit from conditioning exposures, in terms of reduced PTS from high-level noise exposure. Conditioning experiments utilized either UH-60 helicopter noise (1.5 hr/day for 5 days at 90 dB SPL, or 1.5 hr/day for 10 days at 112 dB SPL) or 0.5 kHz OBN (6 hr/day for 5 days at 90-95 dB SPL). Conditioned and control animals were exposed to simulated M16 rifle fire (150 dB peak SPL impulses). The inclusion of UH-60 helicopter noise and M16 rifle fire allowed us to examine sex differences in

susceptibility to noises that soldiers might encounter during training or actual combat (Henselman, 1995).

Experiments conducted during Year 1 of the project showed small but reliable sex differences in auditory sensitivity prior to noise exposure (see Appendix I). In general, female chinchillas had slightly lower high-frequency thresholds, and slightly higher low-frequency thresholds than male chinchillas. but similar input/output functions for evoked potentials and cubic distortion product otoacoustic emissions at suprathreshold input levels. More importantly, data from our control group animals indicated that there are fundamental differences between males and females in their susceptibility to temporary threshold shifts (TTS) and permanent threshold shifts (PTS) caused by exposure to M16 rifle fire. Overall, female chinchillas developed approximately 10 dB more high-frequency PTS, but approximately 5 dB less low-frequency PTS than males. A manuscript describing these results has been submitted to *Ear and Hearing* (see Appendix I). Initial sound conditioning experiments suggested that female and male chinchillas also differ in their ability to develop resistance to PTS. When the conditioning noise was a standard low-frequency noise. males showed little protection from subsequent M16 rifle fire. whereas females showed 10-15 dB protection at most frequencies. When the conditioning noise was helicopter noise at a level of 90 dB for 5 days, males showed only 5 dB or less protection from M16 rifle fire. and females showed no protection in terms of hearing loss. A 10-day series of exposures to UH-60 helicopter noise at 112 dB SPL resulted in no protection for males. and actually potentiated damage from subsequent M16 rifle fire for females. by approximately 10-15 dB.

During Year 2. we have made additional progress in understanding sex differences in susceptibility to noise damage. Experiments using 0.5 kHz OBN and helicopter noise to induce resistance to impulse noise have been completed. Cochleas of noise-exposed animals have been analyzed for hair cell losses. We have also begun to explore the mechanisms underlying noise-induced cochlear damage and possible means for preventing NIHL through pharmacological intervention. Below. we describe sex differences in cochlear hair cell loss following noise exposure. as well as new findings on prevention of NIHL and cochlear damage through sound conditioning and pharmacological intervention strategies.

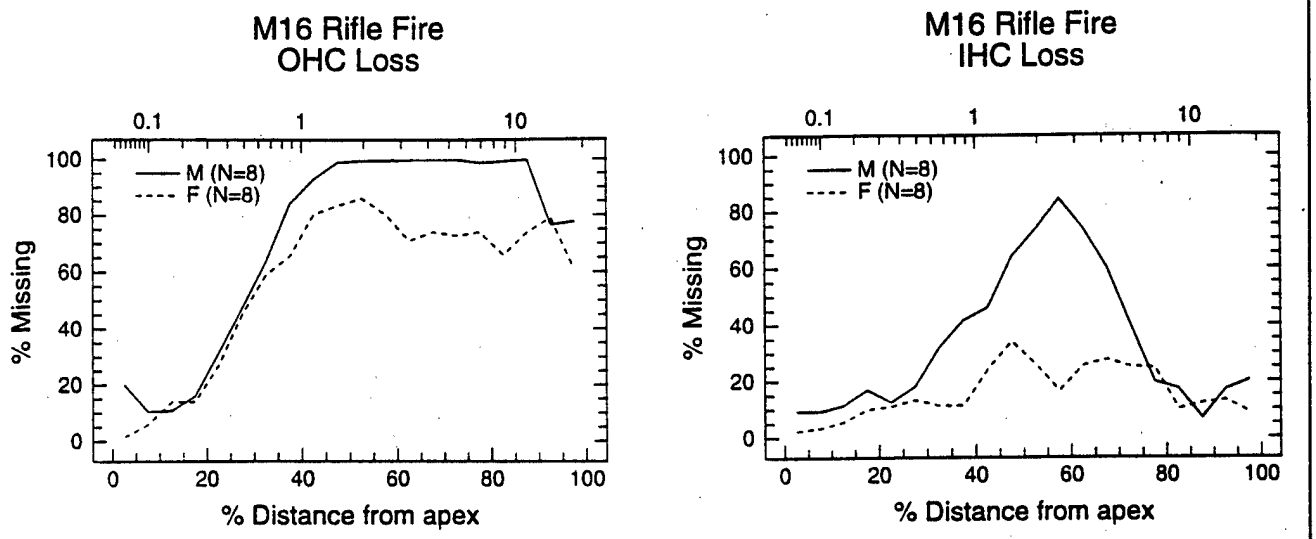
2.2.1. Hair Cell Loss from M16 Rifle Fire and Helicopter Noise

During the past year. we analyzed cochleas of noise-exposed animals whose physiological data were described in the Annual Report for 1996-97. This section will describe the histological results from control animals exposed to impulse noise alone (noise configuration #1) and UH-60 helicopter noise at 90 dB SPL for 5 days or 112 dB for 10 days, followed by impulse noise. Histological results from other groups will be presented in appropriate sections below, along with the results of IC-EVP testing.

2.2.1.1. M16 rifle fire

Sixteen cochleas (8 female. 8 male) of animals exposed to impulse noise alone (noise configuration #1) were examined for hair cell loss. The mean cochleograms are shown in Figure 3. As shown in the left panel of the figure. OHC losses ranged from 70-100% in the basal half of the cochlea for both sexes. However. OHC losses of females (dashed line) were approximately 20% less than OHC losses of males (solid line) in the cochlear regions (>1 kHz) where OHC loss

Fig. 3. Mean cochleograms of male and female chinchillas exposed to M16 rifle fire (150 dB pSPL impulse noise).

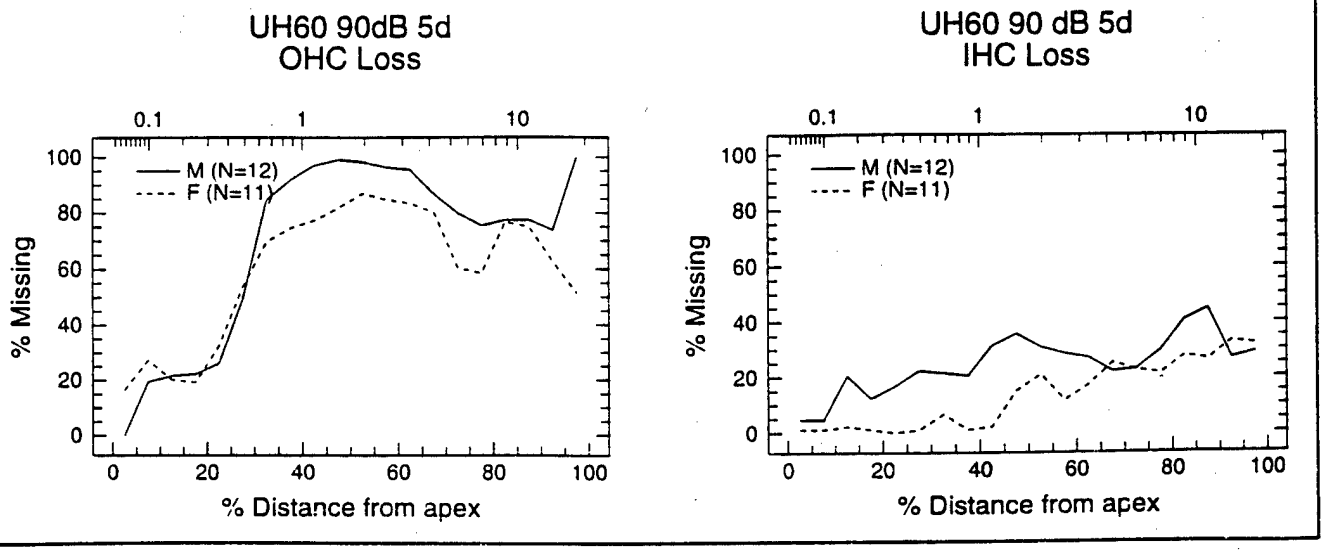


occurred. IHC losses are shown in the right panel of Figure 3. Again, males sustained substantially greater cochlear damage than females. IHC losses for males (solid line) peaked in the 2-3 kHz region of the cochlea, with an average loss of approximately 80%. In contrast, average IHC losses for the females (dashed line) did not exceed 30% in any region of the cochlea.

2.2.1.2 Helicopter noise at 90 dB SPL, 1.5 hr/day for 5 days

Twenty-three cochleas (11 female, 12 male) of animals exposed to UH-60 helicopter noise at 90 dB SPL, 1.5 hr/day for 5 days, were analyzed. The mean cochleograms, shown in Figure 4,

Fig. 4. Mean cochleograms of male and female chinchillas exposed to 90 dB UH-60 Blackhawk helicopter noise for 5 days (1.5 hr/day), followed by M16 rifle fire.



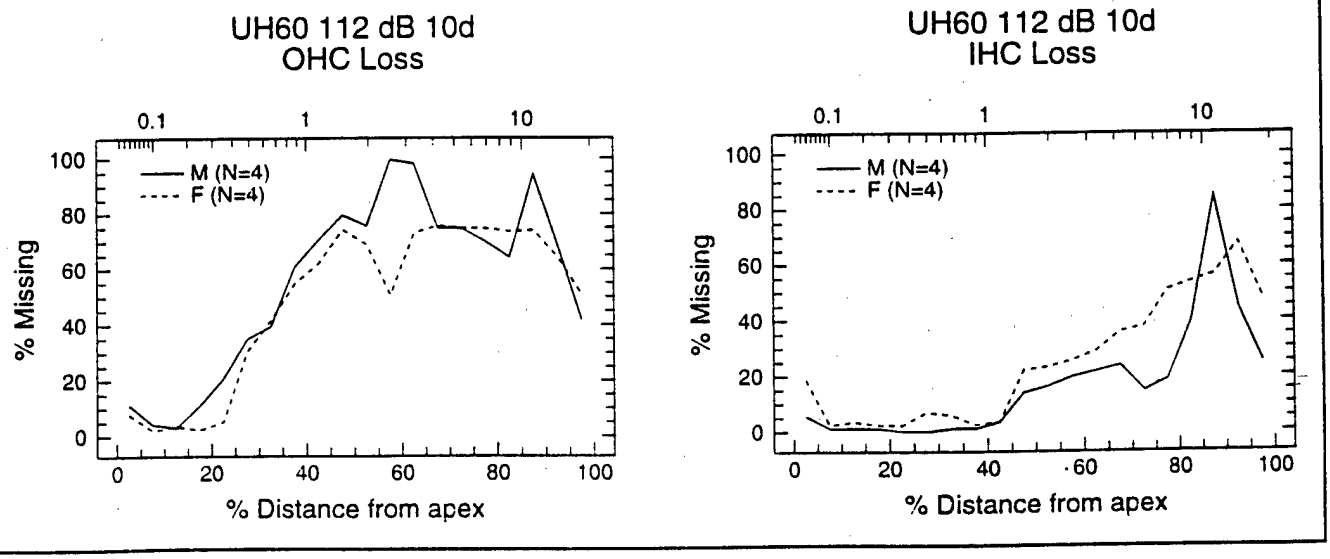
show that hair cells of males were more vulnerable to damage than hair cells of females. OHC losses (left panel) were approximately 10-20% greater for males than females. IHC losses (right panel) were also greater for males than females. Females showed little IHC loss in the apical half of the cochlea, and approximately 20% IHC loss in the basal half. In contrast, males had IHC losses throughout the cochlea, averaging approximately 20% in the apical half, and 30% in the basal half. In the basal-most region of the cochlea, 80-100% distance from the apex, average IHC losses were approximately 30% for females and 40% for males.

When the cochleograms of animals conditioned with 90 dB helicopter noise are compared to those of control animals, it appears that the helicopter noise had two major effects. First, the helicopter noise produced additional damage in the base of the cochlea. Conditioned animals, both males and females, showed approximately 20% greater IHC losses in the 80-100% basal region of the cochlea than their respective controls (Fig. 3). The helicopter noise may have produced basal damage independently, or it may have potentiated the effects of impulse noise. The second effect of 90 dB helicopter noise was to provide some hair cell protection for males in the middle turn of the cochlea. IHC loss in the 2-3 kHz region decreased by approximately 50% after conditioning, and OHC loss in the basal half of the cochlea decreased by approximately 10%. The 10% savings of OHCs in the cochlear base was reflected in the small reduction of high-frequency hearing loss in conditioned males.

2.2.1.3 Helicopter noise at 112 dB SPL, 1.5 hr/day for 10 days

Eight cochleas (4 female, 4 male) of animals exposed to UH-60 helicopter noise at 112 dB SPL, 1.5 hr/day for 10 days, were analyzed. The mean cochleograms are shown in Figure 5. The pattern of hair cell loss, particularly the enhancement of IHC loss in the basal half of the cochlea and the IHC protection for males in the middle turn of the cochlea, was similar to that seen after 90 dB exposure. Again, females had less hair cell damage than males, although the differences after 10 days of 112 dB exposure were relatively small. Compared to 90 dB exposure, which produced approximately 20% and 30% basal IHC losses for females and males,

Fig. 5. Mean cochleograms of male and female chinchillas exposed to 112 dB UH-60 Blackhawk helicopter noise for 10 days (1.5 hr/day), followed by M16 rifle fire.



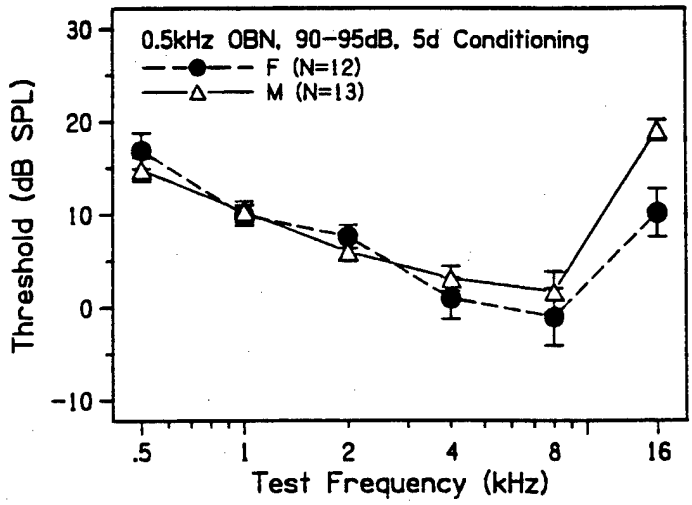
respectively, 112 dB exposure produced basal IHC losses of approximately 40-50%. Thus, the cochleograms indicate that long-term exposure to high-level helicopter noise produces more IHC damage to the base of the cochlea than impulse noise alone. These results are consistent with the physiological results presented in the Annual Report for 1996-1997.

2.2.2. 0.5 kHz OBN Sound Conditioning Experiment

Since the last report was submitted, data have been collected from additional animals, bringing the final sample size for the 0.5 kHz OBN conditioning experiment to 25 ears (12 female, 13 male). Data from an additional male chinchilla were excluded from analysis because this animal's thresholds actually *improved* slightly after impulse noise exposure. We have no explanation for this unusual result, but we are confident that it was not due to experimental error. Since this animal's data would bias the results of comparisons between conditioned and control animals in favor of finding a significant protective effect of conditioning, the data were not included in analysis.

The data presented below show that (a) conditioned females develop less low-frequency PTS than conditioned males, but equivalent high-frequency PTS; (b) conditioned females show slightly less hair cell loss than conditioned males; (c) the 5-day conditioning regimen produced an average of 5-12 dB protection from PTS, with greater protection at low frequencies than at high frequencies; and (d) overall, the 5-day conditioning protocol produced approximately 5-10 dB less protection from PTS than the 10-day protocol used by Henselman et al. (1994).

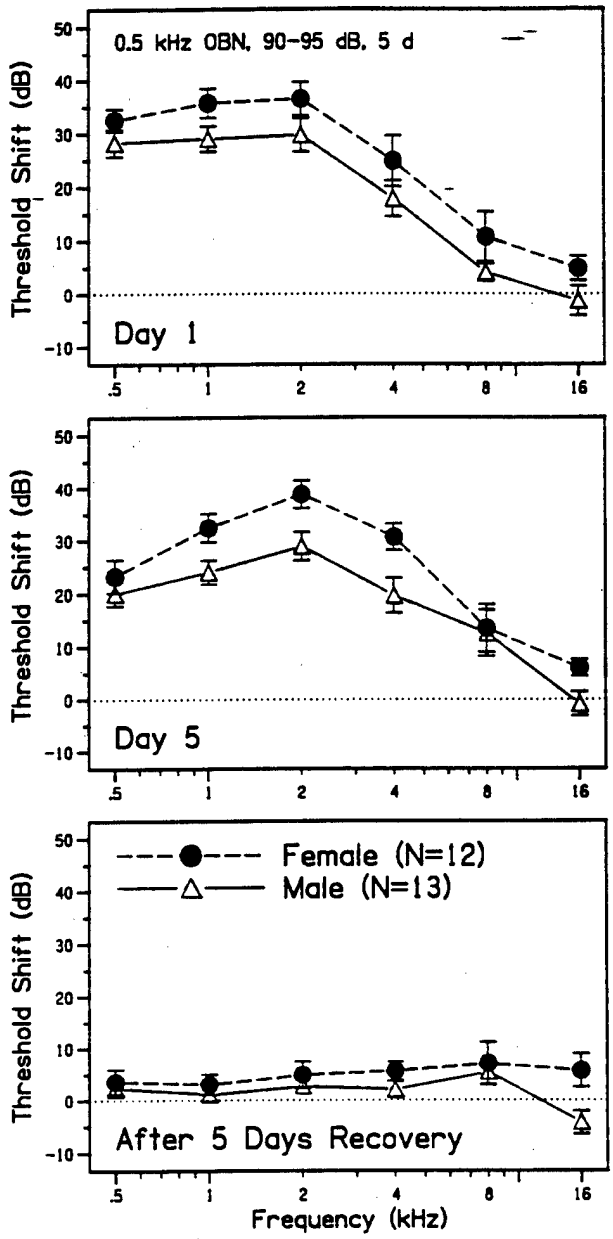
Fig. 6. Pre-exposure thresholds of animals in the 0.5 kHz conditioning group.
(Error bars in this and other line figures represent SEMs.)



2.2.2.1. Noise-induced threshold shifts

Pre-exposure thresholds of animals in the 0.5 kHz conditioning group are shown in Figure 6. Threshold at 16 kHz was approximately 10 dB lower for females than males. Other sex differences were minor, but followed the trend reported for the population (see Appendix I), i.e., lower high-frequency thresholds and higher low-frequency thresholds for females. A two-way (Sex X Frequency) mixed ANOVA revealed a significant Sex X Frequency interaction, $F(5,115)=3.65$, $p=0.004$, due to the reversal of threshold differences between low and high frequencies. Student t-tests at each frequency showed that the difference between females and males at 16 kHz was significant, $t(23)=3.28$, $p=0.003$. Pre-exposure thresholds of the 0.5 kHz conditioning group were not significantly different from those of the control group (see Annual Report for 1996-97 and Appendix I for a full description of the control group).

Fig. 7. Thresholds shifts measured during and after exposure to 0.5 kHz OBN conditioning noise.

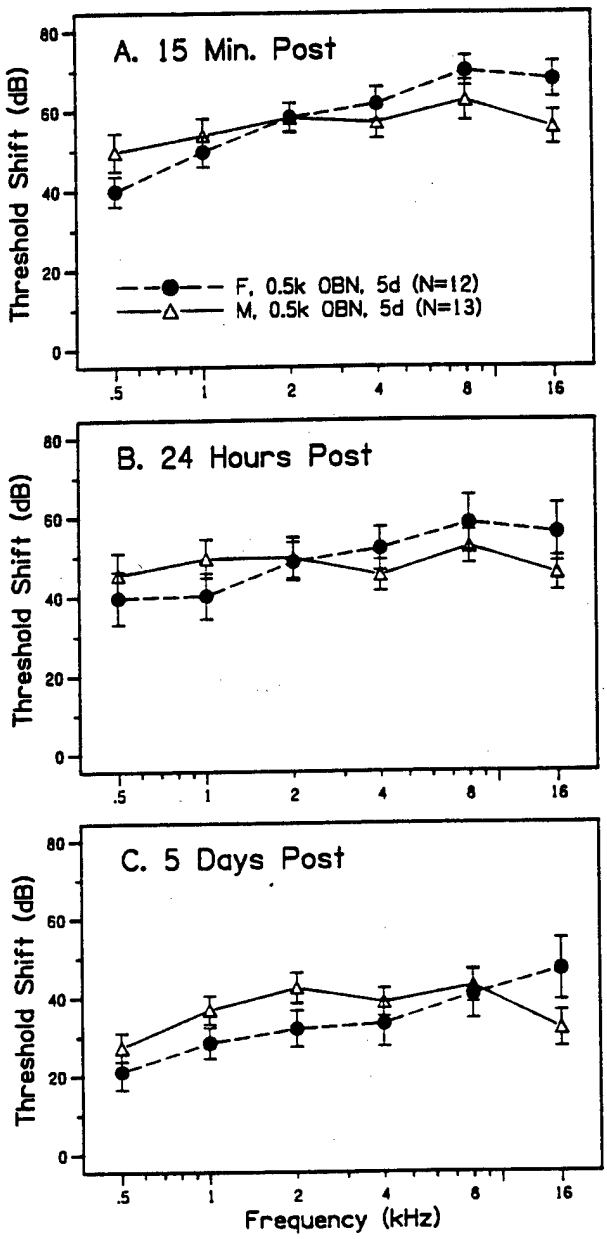


IC-EVP thresholds were measured after the first and last days of conditioning, and again after five days of recovery. Threshold shifts are shown in Figure 7. Conditioning noise produced significant threshold elevations at all frequencies below 16 kHz on Day 1 for both males and females (paired t-tests, all p values <0.04). Two-way (Sex X Time) mixed ANOVAs for TS at each frequency yielded significant main effects of Sex at 1, 2, 4 and 16 kHz and of Time (Day 1, Day 5, 5 days post) at all frequencies except 16 kHz (all p values <0.03). As shown in Figure 7, the main effect of Sex arose because females consistently showed more TS than males, by approximately 5-10 dB during the conditioning exposure.

Paired t-tests were used to follow-up on the significant main effects of Time. These analyses indicated that threshold shift decreased significantly at 0.5 kHz between Day 1 and Day 5 of conditioning for both males, $t(11)=2.73$, $p=0.019$, and females, $t(10)=2.97$, $p=0.014$. The decreases in TS that occurred between the last day of conditioning and 5 days later were significant at all frequencies (all p values <0.04), except at 8 kHz for males. After 5 days of recovery from conditioning, thresholds were within 5 dB of pre-exposure values at all frequencies (Fig. 7, bottom panel).

Animals were exposed to 150 dB impulse noise, and thresholds were measured at 15 min, 24 hr, and 5 days after exposure. Mean TS values at each time are shown in Figure 8. The pattern of TS seen in conditioned animals was very similar to that seen in control animals (see Appendix I), i.e., greater TS at low frequencies for males and greater TS at high frequencies for females. Two-way (Sex X Time) mixed ANOVAs for TS at each frequency revealed a significant Sex X Time interaction at 2 kHz. As shown in Figure 8, the interaction occurred because females

Fig. 8. Threshold shifts of animals in the 0.5 kHz OBN conditioning group after exposure to M16 rifle fire.

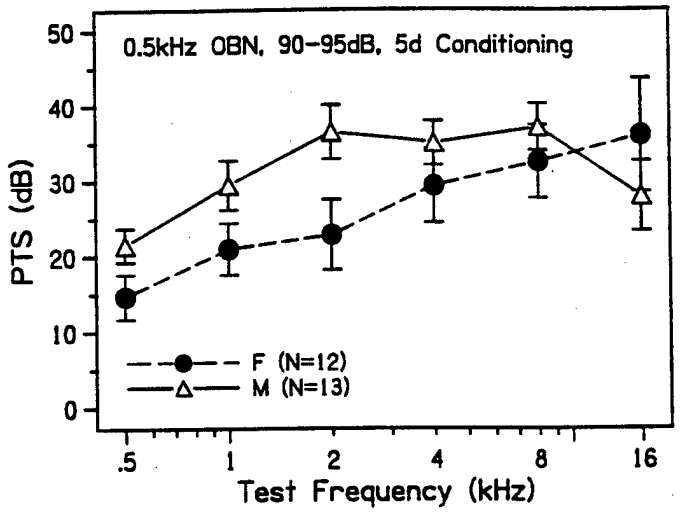


showed more recovery at 2 kHz over time than males. TS at 2 kHz was equivalent for females and males at 15 min and 24 hr, but females showed approximately 10 dB less TS at 5 days post exposure, and 15 dB less PTS than males.

Significant main effects of Time (pre, 15 min, 24 hr, 5 days post, 20 days post) were obtained at each frequency (all p values <0.001). For the follow-up analyses, data were collapsed across sex, since the overall ANOVAs did not yield significant main effects of Sex. At 15 min post-exposure, thresholds were significantly elevated at all frequencies relative to pre-exposure values (all p values <0.001). Mean TS values at 15 min post-exposure ranged from approximately 45 dB at 0.5 kHz, to 65 dB at 8 kHz. Twenty-four hours later, TS values decreased significantly (all p values <0.01) at all frequencies except 0.5 kHz. At this time, TS values ranged from approximately 45 dB at 0.5 kHz to 56 dB at 8 kHz. Thresholds showed further recovery at all frequencies between 24 hr and 5 days post-exposure, and between 5 days post-exposure and PTS (all p values <0.007). PTS was significant at all six frequencies (all p values <0.001).

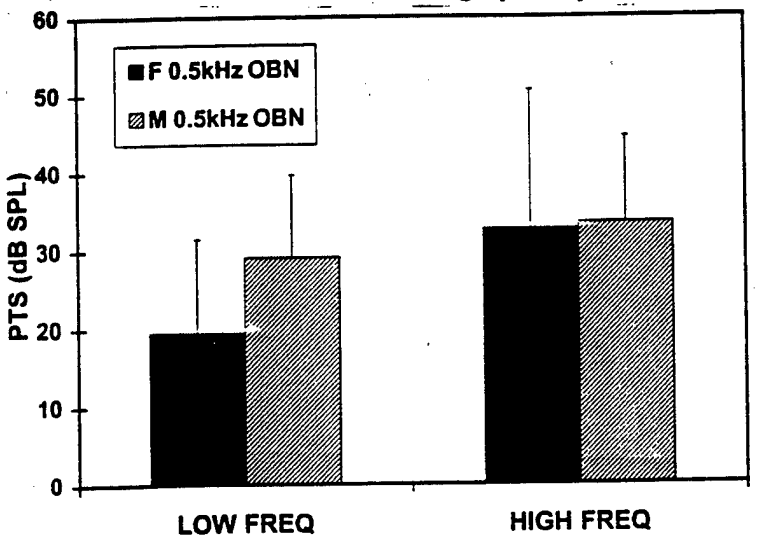
When PTS was assessed, males exhibited 5-15 dB more PTS than females at frequencies below 16 kHz (Fig. 9), and 10 dB less PTS than females at 16 kHz. To evaluate sex differences in PTS, values representing low-frequency PTS (average at 0.5, 1, and 2 kHz) and high-frequency PTS (average at 4, 8 and 16 kHz) were computed. As shown in Figure 10, females showed significantly less low-frequency PTS than males, $F(1,53)=6.14$, $p=0.016$, whereas the difference in high-frequency PTS was not statistically significant.

Fig. 9. PTS of animals conditioned with 0.5 kHz OBN for 5 days before exposure to M16 rifle fire.



To summarize the IC-EVP test results, females had a significantly lower mean threshold at 16 kHz than males prior to exposure (Fig. 6). During conditioning, females consistently showed greater TS than males, but thresholds of both sexes were essentially normal within 5 days after conditioning (Fig. 7). Subsequent exposure to M16 rifle fire resulted in a pattern of TS that resembled that shown by control animals, i.e., more TS at low-frequencies for males, and more TS at high frequencies for females (Fig. 8). When PTS was assessed 20 days after exposure to M16 rifle fire, females showed significantly less low-frequency PTS than males. High frequency PTS was not significantly different between conditioned males and females (Fig. 10).

Fig. 10. Mean low-frequency and high-frequency PTS of animals conditioned with 0.5 kHz OBN.
(Error bars in this and other bar graphs represent SDs.)



2.2.2.2. Noise-induced hair cell losses

Eight cochleas (4 female, 4 male) of animals exposed to the 0.5 kHz conditioning noise were examined for hair cell loss. The mean cochleograms, shown in Figure 11, show slightly less hair cell loss in female cochleas than in male cochleas. Mean OHC losses in the apical half of the cochlea were approximately 30% for females and 40% for males. Average OHC losses in the basal half were approximately 50% for females and 60% for males. The most striking difference is seen in the 1-3 kHz region of the cochlea, where males show approximately 30% more OHC loss than females. Interestingly, this is the frequency region with the largest sex differences in PTS (15 dB

at 2 kHz; see Fig. 9). The cochleograms are consistent with the physiological data described above.

2.2.2.3. Protection from M16 rifle fire

The purpose of exposing animals to the 5-day conditioning regimen was to provide protection from subsequent exposure to M16 rifle fire. A perspective on the success of this approach is provided by Figure 12, which shows differences in PTS between conditioned animals and controls. Figure 12 shows that 0.5 kHz OBN provided up to 18 dB protection for females and up to 10 dB protection for males at individual frequencies. Collapsed across sex, the protective effect was 5 to 12 dB across frequencies, with greater protection at low frequencies than at high frequencies. Figure 13 compares average low-frequency PTS and high-frequency PTS for conditioned and control animals. Protection was apparent for both frequency categories, but only the low-frequency protection was statistically significant, $F(1,53)=6.14$, $p=0.016$.

Fig. 11. Mean cochleograms of female and male chinchillas conditioned with 0.5 kHz OBN before exposure to M16 rifle fire.

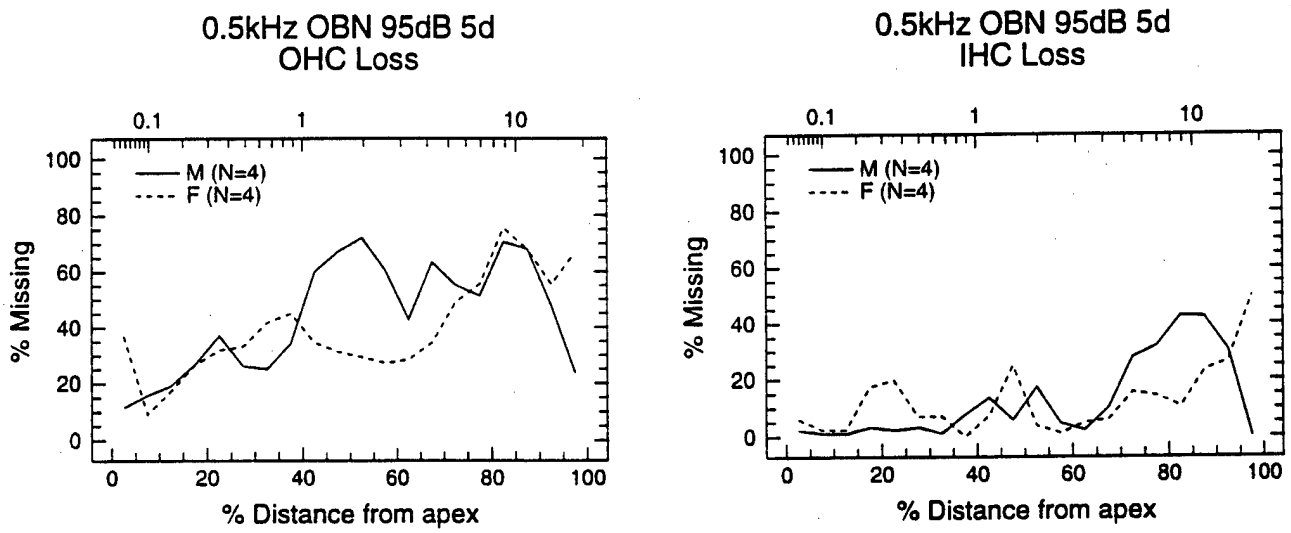


Fig. 12. Differences in PTS between females and males conditioned with 0.5 kHz OBN and their respective controls.

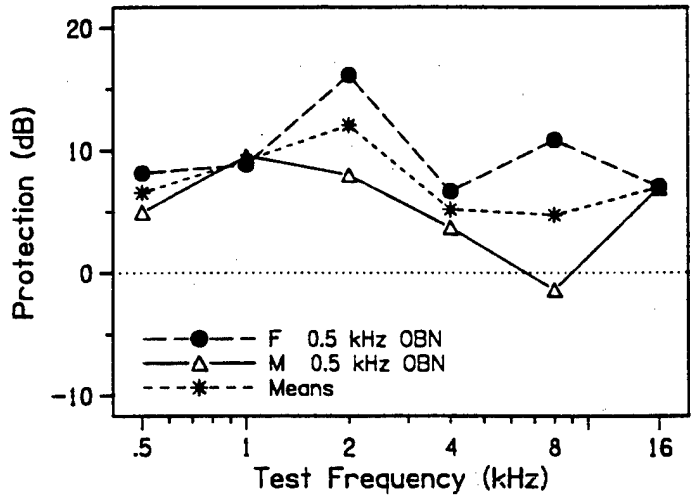


Fig. 13. Average low- and high-frequency PTS of conditioned animals versus controls.

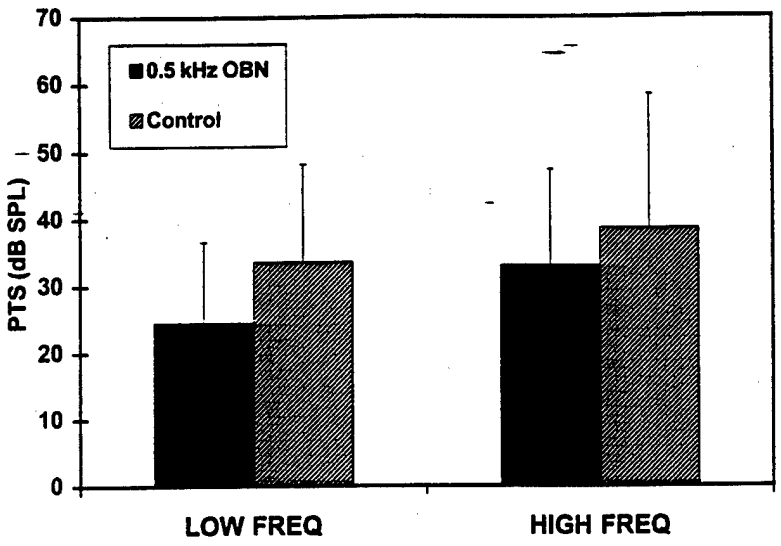


Fig. 14. Average low- and high-frequency PTS according to sex and group.

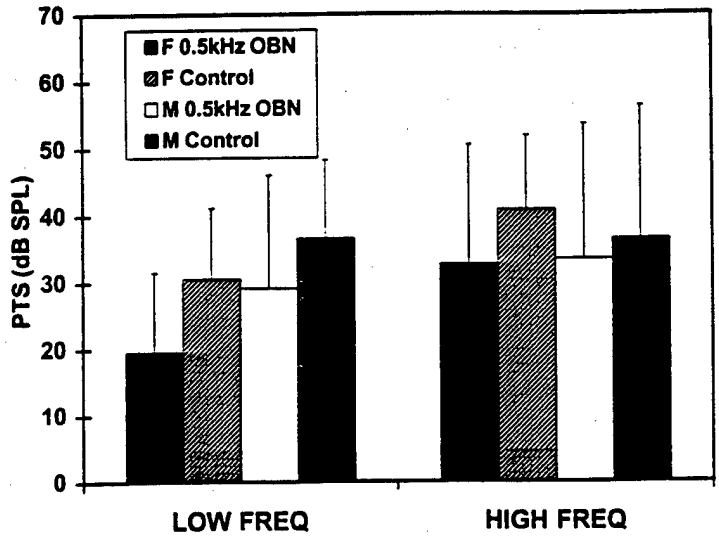


Figure 14 breaks down the protective effect according to sex. Female controls showed less low-frequency PTS but more high-frequency PTS than male controls, as described in our first publication (see Appendix I). Conditioned females and males both showed less PTS than their respective controls. Two-way (Group X Sex) ANOVAs showed significant main effects of Group, $F(1,51)=6.70$, $p=0.12$, and Sex, $F(1,51)=4.70$, $p=0.35$, for low-frequency PTS. Thus, females showed less low-frequency PTS than males, and conditioned animals showed less low-frequency PTS than controls. There were no significant differences between males and females or between conditioned animals and controls in the magnitude of high-frequency PTS.

Cochleograms from conditioned animals (Fig. 10) and controls (Fig. 3) provide further evidence that the low-frequency conditioning was protective. OHC losses were approximately 30% less after conditioning for both females and males, and males also showed a substantial reduction of IHC losses.

The present results clearly indicate a protective effect of the 5-day 0.5 kHz OBN conditioning regimen. In a previous study, Henselman et al. (1994) reported significant protection from impulse noise damage, using a 10-day series of conditioning exposures. Differences in PTS between conditioned animals and controls in Henselman's study were

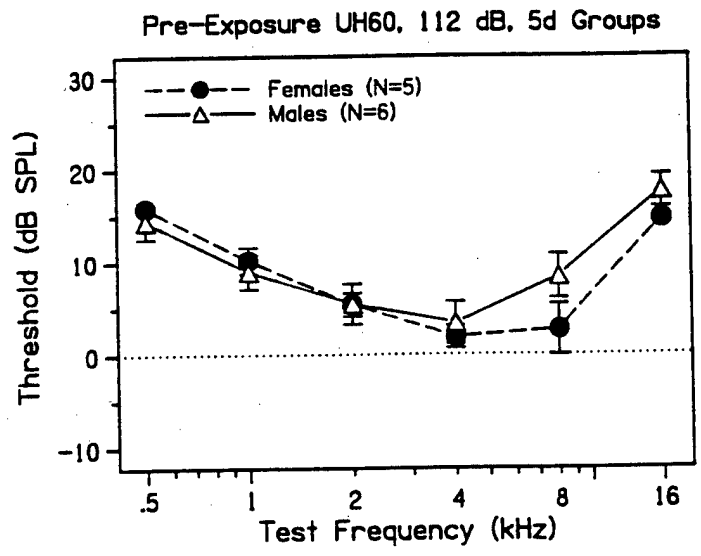
approximately 7-23 dB across frequencies, with the greatest protection at 2 and 4 kHz. Their data were not analyzed on a frequency by frequency basis. However, an examination of their PTS data suggests that the 10-day exposure paradigm resulted in approximately 15 dB protection from low-frequency PTS, and 20 dB protection from high-frequency PTS. Thus, while both conditioning protocols produce significant protection from subsequent exposure to M16 rifle fire, the 10-day regimen provides approximately 5-10 dB more protection than the 5-day regimen used in the current study.

In summary, animals exposed to 0.5 kHz OBN for 6 hr/day for 5 days showed less PTS and hair cell loss following exposure to M16 rifle fire than control animals, particularly at low frequencies. With regard to sex differences, conditioned females showed significantly greater resistance to low-frequency PTS and less OHC loss than conditioned males.

2.2.3. UH-60 Blackhawk Helicopter Noise Sound Conditioning Experiment

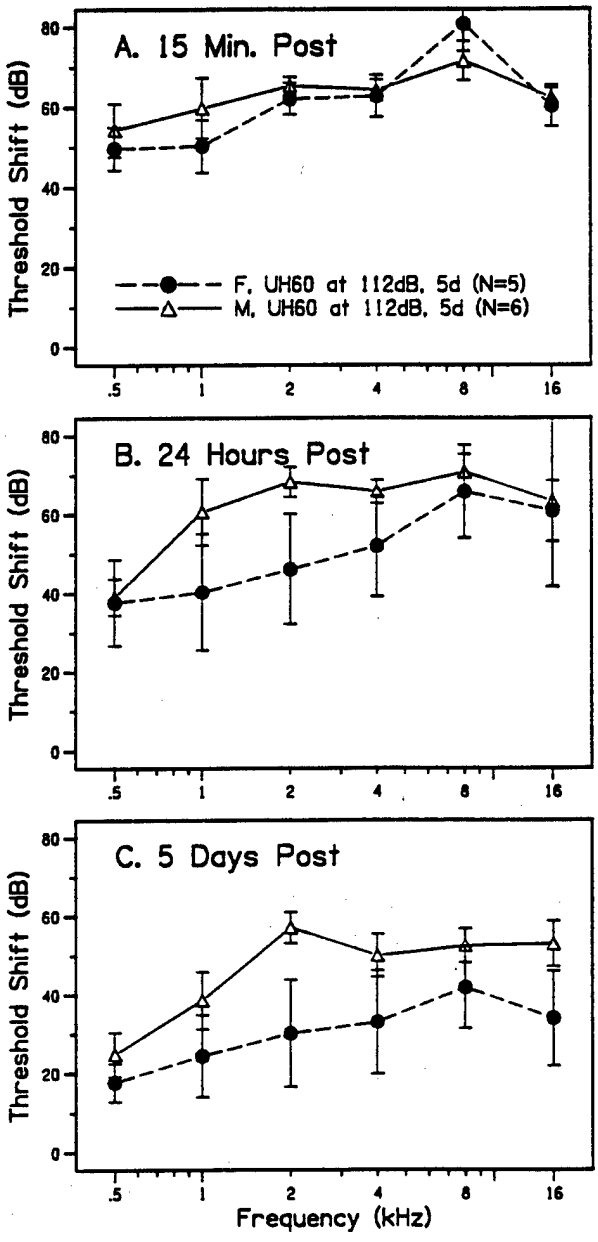
Previous experiments using UH-60 helicopter noise showed minor protection from a 5-day exposure to 90 dB helicopter noise, and either no protection (males) or potentiation of damage (females) from a 10-day exposure to 112 dB helicopter noise. We were interested to know if shortening the duration of the high-level exposure would increase its protective potential and/or decrease its harmful effects. Therefore, an experiment was conducted in which animals were exposed to the helicopter noise at 112 dB dB SPL for 1.5 hr/day for 5 consecutive days. Five days after the conditioning exposure, the animals were exposed to M16 rifle fire.

Fig. 15. Pre-exposure thresholds of animals in the Blackhawk helicopter conditioning group.



The pre-exposure thresholds of animals in the helicopter exposure group are shown in Figure 15. The pattern of sex differences is typical, with females showing slightly higher thresholds at low frequencies, and slightly lower thresholds at high frequencies than males. A three-way (Group X Sex X Frequency) mixed ANOVA did not detect any significant differences between the helicopter group and the control group, nor between females and males. However, the lack of a statistically significant sex difference is most likely due to the small sample size for this conditioning group. This limitation should also be kept in mind when evaluating any negative findings of the statistical tests reported below.

Fig. 16. Thresholds shifts of animals in the helicopter conditioning group after exposure to M16 rifle fire.



Threshold shifts after impulse noise exposure are shown in Figure 16. At 15 min post-exposure, the pattern of TS was similar to that seen in previous exposures, i.e., more low-frequency TS for males, and more high-frequency TS for females. One-way ANOVAs for TS at each frequency detected a significant sex difference at 8 kHz, $F(1,39)=4.38$, $p=0.043$, despite the small sample size. Over the subsequent recovery period, females showed more rapid and complete improvement than males. When PTS was assessed, females showed 5-25 dB less PTS than males across all frequencies (Figure 17). Despite the consistent trend for less PTS in females, no sex differences reached statistical significance.

Differences in PTS between conditioned animals and controls are summarized in Figure 18. The protective effects of helicopter noise differed for females and males. For females, the 5 day helicopter noise exposure provided a small amount of protection (3-10 dB) at all frequencies. In contrast, males showed 5 dB of protection at 0.5 and 1 kHz, and a 5-10 dB potentiation of damage at higher frequencies. Collapsed across sex, the conditioning exposure provided only 3 dB of protection from PTS at low frequencies, and potentiated PTS by 3 dB at high frequencies.

The average low- and high-frequency PTS values are shown in Figure 19 as a function of sex and group. The overall pattern of PTS is remarkably similar to that shown previously for the 0.5 kHz conditioning group (Fig. 10). Again, conditioned females showed less PTS than control females, and females showed less low-frequency PTS than males. With respect to the magnitude of protection, however, it is clear that the helicopter noise has little practical value for preventing subsequent damage from M16 rifle fire.

Fig. 17. PTS of females and males conditioned with helicopter noise before exposure to M16 rifle fire.

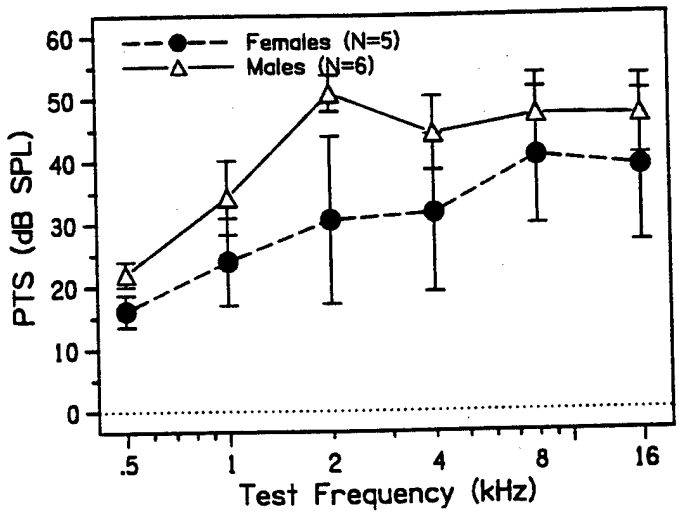


Fig. 18. Differences in PTS between female and male chinchillas in the Blackhawk helicopter conditioning group and their respective controls.

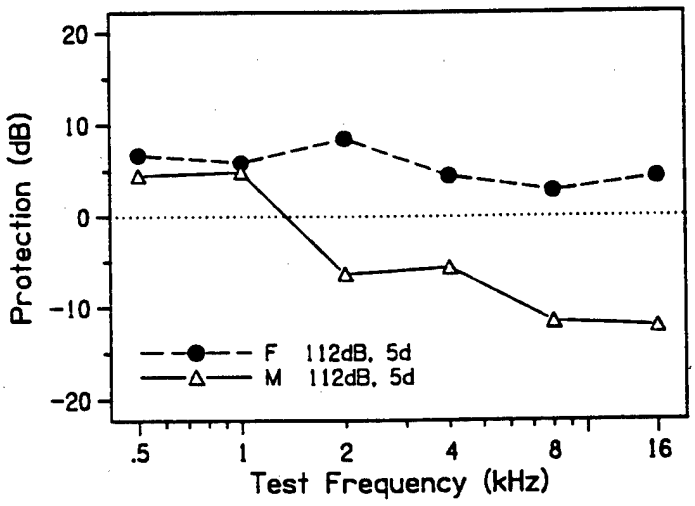
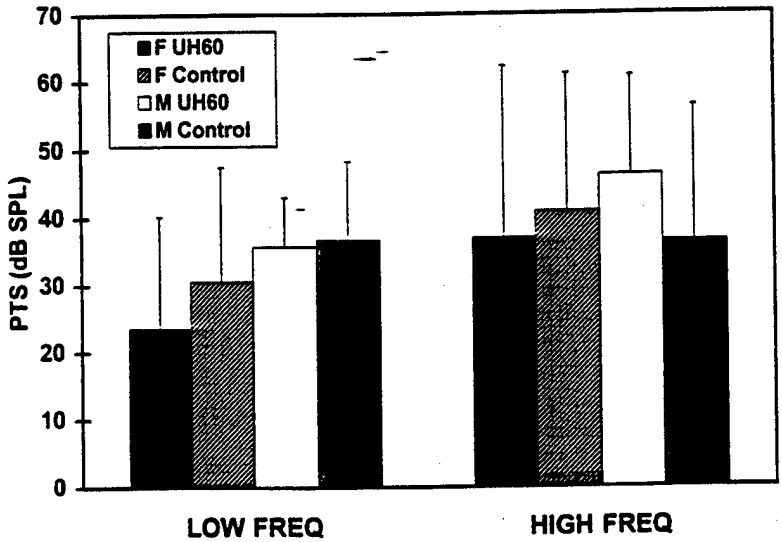


Fig. 19. Average low- and high-frequency PTS according to sex and group.



2.2.4. Pharmacological Intervention for NIHL

Recent studies from our lab and others have implicated reactive oxygen species (ROS) and free radicals as causative agents in NIHL. One line of evidence comes from studies showing that levels of ROS in the cochlea are elevated after noise exposures (Jacono et al., 1996; Yamane et al., 1995; Nicotera et al., 1999). A second line of evidence is provided by studies showing that pharmacological manipulation of antioxidants in the cochlea can reduce NIHL (Hu et al., 1997; Seidman et al., 1993). High levels of noise are likely to create ROS by at least two pathways. First, high levels of noise tax the mitochondrial respiratory process, leading to free radical generation (Radi, et al., 1997; Hyde and Rubel, 1995). Secondly, noise exposures lead to cochlear ischemia, with the consequence of free radical generation at the surface of the stria vascularis (Yamane et al., 1995). Yamane and colleagues exposed guinea pigs to high level noise and assessed blood flow and the presence of superoxide ($0_2^{\bullet-}$) in the stria vascularis. When guinea pigs were sacrificed immediately after the exposure, there was a sharp reduction in cochlear blood flow and a strong presence of $0_2^{\bullet-}$ at the scala media surface of the stria. By two hours after the exposure, cochlear blood flow had returned to normal and most of the free radical activity had subsided.

Recent evidence (Jacono et al. 1998) suggests that conditioning exposures may reduce susceptibility to NIHL by modifying the cochlea's antioxidant defense system. Jacono et al. (1998) showed that noise exposure is associated with significant increases in activity levels of endogenous antioxidant enzymes in the inner ear. The largest increase in enzyme activity levels was found in ears that were exposed to conditioning noise followed by high-level noise. One interpretation of these results is that the increased resistance to noise associated with the sound conditioning paradigm is a reflection of an increased effectiveness of the endogenous antioxidant system.

In our laboratory, Hu et al. (1997) used R-N6-phenylisopropyladenoisine (R-PIA), an adenosine receptor agonist, as a prophylactic treatment before exposure to high-level continuous noise. Briefly, chinchillas were anesthetized and both bullae were opened. A drop of saline was placed on one round window and R-PIA (60 µl of 10^{-3}M solution) was applied to the other round window. One hour after the initial dose of R-PIA, the chinchillas were exposed to a 4 kHz octave band noise at 105 dB for 4 hours. The R-PIA-treated ears recovered faster and more completely and had smaller hair cell lesions than control ears.

In a second complimentary experiment (unpublished data), the same general experimental procedures were used, except the experimental treatment was buthionine sulfoximine (BSO), a drug that inhibits the synthesis of glutathione, one of the most important antioxidants in the cochlea (Hoffman et al., 1988). There were no differences between the ears treated with the small dose of BSO and the saline-treated control ears immediately after the exposure. However, at 4 days post-exposure, differences were apparent in three different measures obtained from separate groups of animals: threshold shifts, glutathione levels in the hair cells (assessed by mercury orange staining of intracellular thiols), and biochemical measures of the specific activity levels of an enzyme necessary for glutathione synthesis. BSO-treated ears had greater TS, reduced glutathione staining in outer hair cells, and lower levels of synthetic enzyme activity than control ears. Despite the clear differences between BSO- and saline-treated ears at 4 days post-exposure, there were no significant differences between ears in either PTS or hair cell loss at 20 days post-exposure. The lack of permanent effects of BSO treatment in this experiment was most likely due to the small dose of BSO used, and the short amount of time it was allowed to penetrate into the cochlea. Nevertheless, the results show that treatment with a drug that inhibits glutathione synthesis in the cochlea alters the pattern of recovery from a traumatic noise exposure. The significant protection afforded by R-PIA and the altered pattern of recovery with BSO are consistent with the hypothesis that NIHL is partially related to the action of toxic free radicals.

The results described above raise the possibility that NIHL can be reduced or prevented by increasing the levels of endogenous antioxidant enzymes of the cochlea through certain prophylactic sound conditioning exposures, or by direct drug intervention. In the following pilot experiment, we looked at the effectiveness of R-PIA in reducing NIHL from impulse noise.

2.2.4.1. Protection from M16 rifle fire with R-PIA

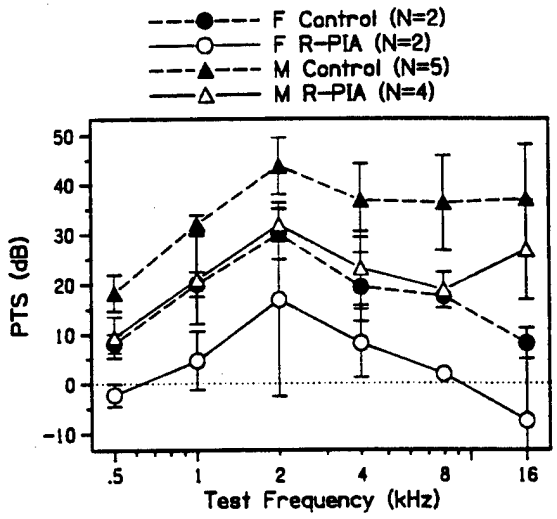
The effectiveness of R-PIA in protecting the ear from the damaging effects of continuous noise prompted us to examine the issue of whether R-PIA could also attenuate hearing loss and hair cell loss caused by impulse noise. The results of the pilot experiment described here are very exciting, as they suggest that pre-treatment with R-PIA renders the ear more resistant to the mechanical damage associated with impulse noise. Moreover, they suggest that there may be sex differences in the effectiveness of R-PIA in the prevention of cochlear damage.

As described in the Methods section, R-PIA was applied to the round window membrane of the right ear, and saline or nothing was applied to the round window membrane of the left ear. Pre-exposure thresholds were measured, then animals were exposed to impulse noise (noise configuration #2). Data from the left ears show the response of the ear to the impulse noise alone, whereas data from the right ears of the same animals show the effects of R-PIA in

reducing damage. Due to the small sample size (2 females, 5 males), statistical tests were not performed. However, the trends in the data are strong, and clearly argue for further study.

Pre-exposure thresholds were similar for males and females. Females had slightly higher thresholds than males at 0.5-4 kHz by 5-10 dB. and equivalent thresholds at 8 and 16 kHz. Figure 20 shows PTS measured 20 days after exposure to impulse noise (noise configuration #2). Three aspects of the results are particularly noteworthy. First, females consistently showed less PTS than males. For both R-PIA-treated ears and control ears, sex differences were on the order of 15-35 dB. Second, pre-treatment with R-PIA clearly reduced PTS. R-PIA pre-treatment resulted in a 10-15 dB reduction of PTS across frequencies. Third, R-PIA pre-treatment was equally effective for females and males. That is, the magnitude of protection was similar for both sexes.

Fig. 20. PTS measured 20 days after impulse noise exposure.



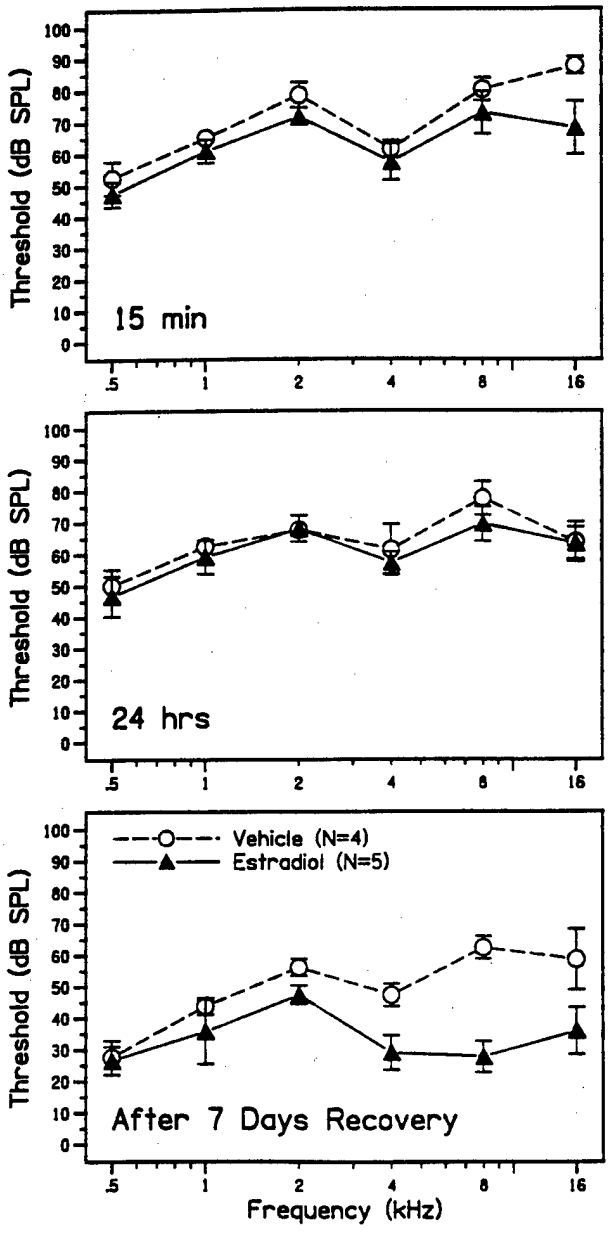
The protection afforded by the R-PIA treatment may be due to several factors, as R-PIA has multiple effects (Maggiwar et al., 1994). It is an adenosine agonist, an inhibitor of glutamate production, and a promoter of blood flow through stimulation of nitric oxide (NO) production. Each of these effects can influence the degree of hearing loss caused by exposure to noise. For example, the activation of the adenosine receptor can lead to an increase in glutathione and a resultant decrease in toxic ROS (Ford et al., 1997; Bobbin et al., 1995). By inhibiting glutamate production, R-PIA can reduce glutamate excitotoxicity at the synapse between the inner hair cells and the afferent nerve fibers that innervate them. Finally, R-PIA may partially counteract the cochlear ischemia that is produced by acoustic overexposure by promoting local blood flow through stimulation of NO production. Future studies should target the specific mechanism of R-PIA induced protection in order to maximize the protective effects.

2.2.4.1. Protection from M16 rifle fire with 17-β-estradiol

Like R-PIA, steroid hormones can act through many different routes, each of which could be important for modulating susceptibility to NIHL. Steroids such as estrogen (estradiol) can potentiate the activity of GABA, a ubiquitous inhibitory neurotransmitter of the central nervous system; they can affect neuronal activity via changes in cellular neurochemistry and morphology; they can act on cell membranes to alter permeability to neurotransmitters, precursors and receptor functioning; they can act directly as antioxidants; and they can influence the bioactivity of other antioxidants and blood flow promoters such as NO (Arnal et al., 1996; Ayres et al.,

1996; Behl et al., 1995; Chadwick and Widdows, 1990; Goodman et al., 1996; Romer et al., 1997; Ruiz-Larrea et al., 1994).

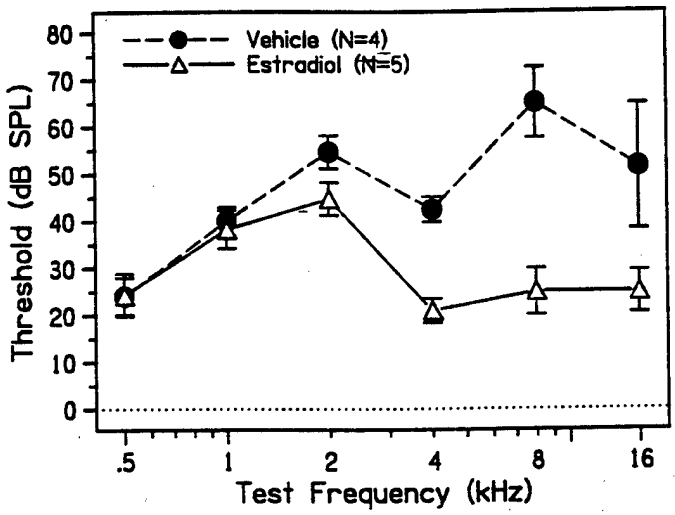
Fig. 21. Thresholds shifts measured after exposure to M16 rifle fire.



In the following pilot study, we examined the effects of the ovarian hormone, 17-β-estradiol, on hearing loss from impulse noise (noise configuration #2). Chinchillas in the estradiol group were given daily injections of estradiol in olive oil for two weeks before exposure, while animals in the control group received injections of the olive oil vehicle alone. Thresholds measured during the course of hormone treatment were not different from those measured before treatment, indicating that short-term estradiol treatment has no direct effect on auditory sensitivity.

Threshold shifts measured 15 min to 7 days after exposure to M16 rifle fire are shown in Figure 21. There was little difference between the two groups immediately after exposure. Over the next seven days, however, estradiol-treated animals recovered faster and more completely at high frequencies than control animals. When PTS was assessed (Fig. 22), estradiol-treated animals showed 20-40 dB less PTS at high frequencies (4, 8 and 16 kHz) than controls. These are very dramatic differences, and are considerably larger than those typically seen in sound conditioning experiments. Although the results are preliminary and based on only 9 animals (5 treated, 4 control), they strongly suggest that estradiol can facilitate recovery from impulse noise exposure. It is interesting to speculate that levels of endogenous steroid hormones may be an important factor in determining an individual's susceptibility to NIHL.

Fig. 22. PTS of animals in the estradiol and control groups.



2.3. Recommendations

In the original proposal for this project, the following questions were listed as targets of study during Years 1 and 2 (Phase I) of the project:

1. Are there consistent differences between male and female chinchillas in hearing sensitivity?

2. How do female chinchillas compare to males in their susceptibility to NIHL caused by impulse noise typical of noises found in military environments?

3. Do conditioning exposures produce equivalent protection for male and female chinchillas against damage and hearing loss caused by subsequent high-level noise exposure?

The five experiments designed to answer the above questions have been completed on schedule. The experiments conducted in Year 2 expanded the findings from Year 1, and added new insights into the mechanisms underlying NIHL. All of the technical objectives for Years 1 and 2 have been met, and all of the specific tasks listed for Year 2 have been completed. In the following section, our original technical objectives for Years 1 and 2 are presented in italicized text. Statements pertaining to our accomplishment of the objectives are presented in regular text.

Technical Objective #1: *Examine auditory sensitivity before, during and after noise exposure as a function of sex (male versus normal female chinchillas) and conditioning exposure.*

[NOTE: The complete protocol, from surgical preparation to final testing, will require approximately 2 months for each group of 4-6 animals. Animals will be prepared, tested, and exposed to noise in groups of 4-6 at a time. Some overlap in exposures and recovery occurs, so

that Phase I of the project will require approximately 22 months for all exposures and data collection to be completed.]

The five experiments conducted during Years 1 and 2 have provided essential information regarding sex differences in (a) basic auditory sensitivity, (b) susceptibility to noise-induced hearing loss and cochlear damage, and (c) the ability to benefit from prophylactic sound conditioning exposures.

Task 1: *Months 1-6: Surgically prepare first 20 male and 20 female chinchillas for auditory evoked potential (AEP) recordings. [Note: Approximately 4 animals can be surgically prepared in a week. However, because space in the animal colony is limited and the noise exposure booth must be scheduled for use, there may be some weeks when no new animals can be introduced into the experiment.]*

Months 8-14: Surgically prepare 40 more chinchillas.

A total of 96 chinchillas were surgically prepared for IC-EVP testing during the past two years. The next group of animals is scheduled for surgery in early November. Phase I of the project was completed with fewer animals than originally estimated, primarily due to an exceptionally high survival and completion rate during Year 1. We attribute the unusual surgical success rate to the outstanding surgical skills of X.Y. Zheng, the Research Scientist working on the project.

Task 2: *Months 1-18: Perform preliminary hearing tests (3 AEP and 3 CDP tests) on surgically prepared animals.*

Pre-exposure IC-EVPs were obtained from all animals. Most animals in Year 1 and some animals in Year 2 were also tested for CDPs. Each animal was tested three times, and the measurements were averaged for stable baseline estimates of IC-EVP thresholds and CDP input/output functions. In Year 2, one of the signal processing boards in our CDP system malfunctioned, and we were unable to collect reliable CDPs in later experiments. The DSP boards are no longer available from the manufacturer, and the system cannot be repaired. We are currently looking into the feasibility of replacing our system with new equipment. In the meantime, we do not consider the lack of CDP data to be a problem. While we would have preferred to have CDP measures as well as IC-EVP measures, recent studies from our lab and others have shown that the two measures provide essentially the same information about the functional status of the outer hair cell system (McFadden and Campo, 1998; McFadden et al., 1998b). Since the IC-EVP measures are adequate to assess the functional status of the OHCs, no experiments were compromised by our inability to measure CDPs in Year 2.

Task 3*: Months 1-7: Expose 10 males and 10 females to 10 days of conditioning noise followed 5 days later by exposure to impulse noise. Test hearing during and after exposures (i.e., on Days 1, 5, and 10 of conditioning, 5 days after conditioning, and at 15 min., 24 hr, and 10 days after high-level exposure). Expose 10 male and 10 female control animals to impulse noise alone; test at 15 min, 24 hr, and 5 days post-exposure.*
All animals will be tested three more times after 30 days of recovery from high-level noise, then their cochleas will be collected for histological analysis.

Months 9-22: Expose animals to appropriate conditioning and high-level noises; test hearing during and after exposure.

Data collection and analyses are complete for the control group and the four sound conditioning groups. Conditioned animals were tested during and after conditioning. Conditioned and control animals were exposed to M16 rifle fire, and tested at 15 min, 24 hr, 5 days, and 20-30 days after high-level exposure. Cochleas were collected for histological analyses, and hair cell counts were performed during Year 2.

Task 4: *Months 1-22: On-going data entry and analysis.*
Data collection will be completed for the 10-day helicopter noise conditioning group and the impulse noise control group by Month 11.
Data collection for the two 5-day helicopter noise conditioning groups will be completed by Month 19.
Data collection for the low-frequency conditioning noise group will be completed by Month 22.

Data collection has been completed for all three helicopter noise conditioning groups, the 0.5 kHz OBN conditioning group, and the impulse noise control group.

Task 5: *Month 11: Preparation of data for presentation at professional meeting, describing sex differences in the response of the auditory system to high-level impulse noise.*

The PI attended the 1998 Midwinter Meeting of the Association for Research in Otolaryngology (ARO) in St. Petersburg Beach, FL, in February, 1998. At the meeting, the PI conferred with several prominent researchers in field, including Dr. Barbara Canlon from Karolinska Institutet in Sweden and Dr. James F. Willott of Northern Illinois University, about the on-going research.

Task 6: *Month 12: Prepare detailed annual report to summarize project findings and progress.*

The annual report for 1996-1997, summarizing experiments performed during Year 1 of the project, was submitted on time and approved.

Task 7: *Month 23: Preparation of data for presentation at professional meeting, detailing sex differences in susceptibility to NIHL and development of resistance to NIHL.*

An abstract describing sex differences in susceptibility to noise-induced hearing loss has been submitted to ARO (see Appendix II). The data will be formally presented at the upcoming ARO annual meeting in February, 1999.

Task 8: *Month 23-24: Preparation of manuscript for submission to a peer-reviewed journal, detailing sex differences in susceptibility to impulse noise damage and resistance developed by 5 or 10 days of conditioning with helicopter noise.*

A manuscript describing sex differences in basic auditory sensitivity and susceptibility to impulse noise has been submitted to *Ear and Hearing*. The manuscript has been reviewed once, and the revised version was submitted on September 14, 1998. We expect that the manuscript will be published in December, 1998 or January, 1999. A second manuscript describing sex differences in the ability to develop resistance to noise through sound conditioning is currently in preparation.

Task 9: *Month 24: Submission of second annual report to summarize project findings and progress.*

The current report summarizes data collected and/or analyzed during Year 2 of the project. The report summarizes the results of the five experiments proposed for Phase I (Years 1 and 2) of the project, including cochlear histology. In addition, the report describes two pilot studies utilizing pharmacological agents (R-PIA and 17-β-estradiol) to prevent NIHL. The 17-β-estradiol experiment is the first step toward fulfilling the technical objectives for the final year of the project.

Technical Objective #2: *Examine differences in sensitivity before, during and after noise exposure as a function of hormonal status.*

A pilot study has been completed in which chinchillas were treated with daily subcutaneous injections of 17-β-estradiol, an ovarian steroid hormone, prior to impulse noise exposure. The preliminary results show remarkable protection from NIHL in estradiol-treated animals. These are particularly exciting results, as this is the first instance in which an endogenous steroid hormone has been shown to reduce NIHL. Additional studies will be conducted during Year 3 to expand on our initial findings, as outlined in our original proposal.

3. Conclusions

The purpose of Phase I of this project was to investigate sex differences in auditory sensitivity, susceptibility to NIHL caused by simulated M16 rifle fire, and the ability to develop resistance to NIHL using sound conditioning procedures. Hearing function was assessed by measuring sound-evoked electrical activity from the inferior colliculus, and cochlear damage was assessed by determining the number of missing inner and outer hair cells in the organ of Corti.

Experiments completed during Year 1 led to the following conclusions:

1. Chinchillas exhibit small, but consistent sex differences in auditory sensitivity, with females showing slightly better thresholds at high frequencies, but slightly worse thresholds at low frequencies than males. This pattern matches the pattern described for humans, suggesting that the chinchilla may be a good model for exploring the anatomical and physiological bases of sex/gender differences.

2. Chinchillas exhibit sex differences in their susceptibility to hearing loss caused by exposure to high-level (150 dB peak SPL) impulse noise (simulated M16 rifle fire). Female chinchillas

develop less hearing loss at low frequencies, but more hearing loss at high frequencies than males.

3. Long-term exposure to high-level (112 dB SPL) UH-60 Blackhawk helicopter noise potentiates hearing loss from simulated M16 rifle fire in females.

4. Exposure to UH-60 Blackhawk helicopter noise at 90 dB SPL (i.e., a level that might be experienced by a soldier wearing PPDs while riding in the cabin of the helicopter) neither increases nor decreases susceptibility to hearing loss caused by a subsequent exposure to simulated M16 rifle fire. There are no sex differences in this regard.

Experiments completed during Year 2 lead to the following additional conclusions:

1. Overall, cochleas of males appear to be more vulnerable to noise-induced hair cell loss than cochleas of females. Males showed much greater IHC and OHC loss after exposure to M16 rifle fire than females. Animals exposed to UH-60 Blackhawk helicopter noise followed by M16 rifle fire had less hair cell loss than control animals exposed to only M16 rifle fire, but again, males tended to have more hair cell loss than females.

2. Chinchillas conditioned with low-frequency noise (0.5 kHz OBN at 90-95 dB) prior to exposure to M16 rifle fire develop significantly less low-frequency PTS than control animals exposed only to the M16 rifle fire. Conditioned females show significantly less low-frequency PTS and slightly less hair cell loss than conditioned males.

3. Although a 5-day low-frequency conditioning regimen produces significant protection from PTS, the magnitude of protection is 5-10 dB less than that derived from a 10-day conditioning regimen.

4. Exposure to Blackhawk helicopter noise for 5 days at 112 dB SPL (1.5 hr/day) was relatively ineffective in protecting either males or females from subsequent exposure to M16 rifle fire.

5. Initial studies using R-PIA suggest that it may be beneficial in reducing PTS from M16 rifle fire. The magnitude of protection appears to be similar for male and female chinchillas.

6. Initial studies using 17-β-estradiol show a large degree of protection from M16 rifle fire in treated animals. The results raise the interesting possibility that susceptibility to NIHL may be partially related to endogenous levels of steroid hormones.

The results from the experiments described here have important practical and theoretical implications. From a theoretical standpoint, they provide a much-needed perspective on the role of sex-related factors on normal physiology and function, thereby increasing our understanding of basic auditory system physiology. Gender differences have frequently been reported in humans, but the basis for these differences has remained elusive. Often, sex differences have been attributed to differences in noise exposure history, as males are more often engaged in occupational and recreational activities that involve high-level noise. However, our finding of parallel sex differences in chinchillas argue for more fundamental, inherent differences. These

may involve acoustical properties of the external and middle ears, differences in the length of the cochlea, and/or biochemical and hormonal factors. Further research with the chinchilla model will be useful for determining the relative importance of each of these factors.

From a practical standpoint, the results of our experiments with chinchillas can aid in designing programs and procedures for reducing hearing loss in military personnel who are exposed to traumatic noise. Overall, the data point to important sex differences in the response of the cochlea to high-level impulse noise and sound conditioning, which could have important implications for military assignments and hearing conservation programs. In addition, our pilot data on pharmacological intervention have exciting implications for the prevention of NIHL.

4. References

Arnal, J.F., Clamens, S., Pechet, C., Negre-Salvayre, A., Allera, C., Girolami, J.-P., Salvayre, R. and Bayard, F. (1996) Ethinylestradiol does not enhance the expressiion of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. *Proc. Natl. Acad. Sci. USA* 93, 4108-4113.

Ayres, S., Tang, M. and Subbiah, M.T.R. (1996) Estradiol-17β as an antioxidant: Some distinct features when compared with common fat-soluble antioxidants. *J Lab Clin Med* 128, 367-375.

Behl, C., Widmann, M., Trapp, T. and Holsboer, F. (1995) 17-β estradiol protects neurons from oxidative stress-induced cell death *in vitro*. *Biochem Biophys Res Comm* 216, 473-482.

Berger, E.H., Royster, L.H. and Thomas, W.G. (1978) Presumed noise-induced permanent threshold shift resulting from exposure to an A-weighted Leq of 89 dB. *J Acoust Soc Am* 64, 192-197.

Bobbin, R.P., Fallon, M., LeBlanc, C. and Baber, A. (1995) Evidence that glutathione is the unidentified amine (Unk 2.5) released by high potassium into cochlear fluids. *Hear Res* 87, 49-54.

Bohne, B.A., Gruner, M.M. and Harding, G.W. (1990) Morphological correlates of aging in the chinchilla cochlea. *Hear Res* 48, 79-91.

Campo, P., Subramaniam, M. and Henderson, D. (1991) Effect of "conditioning" exposures on hearing loss from traumatic exposure. *Hear Res* 55, 195-200.

Chadwick, D. and Widdows, K. (Eds.) Steroids and Neuronal Activity. New York: John Wiley & Sons

Clark, J.D. (1984) Biology and diseases of other rodents. In: J.G. Fox, B.J. Cohen and F.M. Lowe (Eds.), *Laboratory Animal Medicine*. New York: Academic Press Inc.

Dancer, A., Buck, K., Parmentier, G. and Hamery, P. (1998). The specific problems of noise in military life. *Scand Audiol* 27, 123-130.

Dancer, A. and Franke, R. (1986) Effects of weapon noise on hearing. In: R.J. Salvi, D. Henderson, R.P. Hamernik and V. Colletti (Eds.), *Basic and Applied Aspects of Noise-Induced Hearing Loss*. New York: Plenum Press.

Danielson, R., Henderson, D., Gratton, M. A., Bianchi, L. and Salvi, R. (1991) The importance of "temporal pattern" in traumatic impulse noise exposures. *J Acoust Soc Am* 90, 209-218.

Ford, M.S., Maggiwar, S.B., Rybak, L.P., Whitworth, C. and Ramkumar, V. (1997) Expression and function of adenosine receptors in the chinchilla cochlea. *Hear Res* 105, 130-140.

Gallo, R. and Glorig, A. (1964) Permanent threshold shift changes produced by noise exposure and aging. *J Ind Hyg* 25, 237-245.

Goodman, Y., Bruce, A.J., Cheng, B. and Mattson, M.P. (1996) Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid β-peptide toxicity in hippocampal neurons. *J Neurochem* 66, 1836-1844.

Greenwood, D.D. (1990) A cochlear frequency-position function for several species—29 years later. *J Acoust Soc Am* 87, 2592-2604.

Heffner, R.S. and Heffner, H.E. (1991) Behavioral hearing range of the chinchilla. *Hear Res* 52, 13-16.

Henderson, D., Spongr, V.P., Subramaniam, M. and Campo, P. (1994) Anatomical effects of impact noise. *Hear Res* 76, 101-117.

Henderson, D., Hamernik, R.P. and Sitler, R.W. (1974) Audiometric and histological correlates of exposure to 1-ms noise impulses in the chinchilla. *J Acoust Soc Am* 56, 1210-1221.

Henderson, D., Hamernik, R.P., Salvi, R.J. and Ahroon, W.H. (1983) Comparison of auditory-evoked potentials and behavioral thresholds in normal and noise-exposed chinchillas. *Audiol* 22, 172-180.

Henselman, L.W. (1995) Effect of "conditioning" on hearing loss from military noise exposures. Unpublished Doctoral Dissertation, State University of New York at Buffalo.

Henselman, L.W., Henderson, D., Shadoan, J., Subramaniam, M., Saunders, S. and Ohlin, D. (1995) Effects of noise exposure, race, and years of service on hearing in U.S. Army soldiers. *Ear Hear* 16, 382-391.

Henselman, L.W., Henderson, D., Subramaniam, M. and Sallustio, V. (1994) The effect of 'conditioning' exposures on hearing loss from impulse noise. *Hear Res* 78, 1-10.

Hoffman, D.W., Whitworth, C.A., Jones-King, K.L. and Rybak, L.P. (1988) Potentiation of ototoxicity by glutathione depletion. *Annals Otol Rhinol Laryngol* 97, 36-41.

Hu, B.H., Zheng, X.Y., McFadden, S.L., Kopke, R. and Henderson, D. (1997) R-PIA attenuates noise-induced hearing loss in the chinchilla. *Hear Res* 113, 198-206.

Hyde, G.E. and Rubel, E.W. (1995) Mitochondrial role in hair cell survival after injury. *Otolaryngol-Head Neck Surg* 113, 530-40.

Jacono, A., Kopke, R., Vungmyster, L., Steinman, H., Rowe, J., Hu, B.H., Van De Water, T.R. and Henderson, D. (1996) Changes in cochlear antioxidant enzyme activity levels after conditioning noise exposures in the chinchilla. *Assoc Res Otolaryngol* Abstr.

Maggirwar, S.B., Dhanraj, D. and Samani, S.M. (1994) Adenosine acts as endogenous activator of the cellular antioxidant defense system. *Biochem Biophys Res Comm* 201, 508-515.

McFadden, S.L. and Campo, P. (1998) Cubic distortion product otoacoustic emissions in young and aged chinchillas exposed to low-frequency noise. *J Acoust Soc Am* (in press).

McFadden, S.L., Campo, P., Quaranta, N. and Henderson, D. (1997) Age-related decline of auditory function in the chinchilla (*Chinchilla laniger*). *Hear Res* 111, 114-126.

McFadden, S.L., Campo, P., Ding, D.L. and Quaranta, N. (1998) Effects of noise on inferior colliculus evoked potentials and cochlear anatomy in young and aged chinchillas. *Hear Res* 117, 81-96.

McFadden, S.L., Kasper, C., Ostrowski, J., Ding, D.L. and Salvi, R.J. (1998) Effects of inner hair cell loss on inferior colliculus evoked potential thresholds, amplitudes and forward masking functions in chinchillas. *Hear Res* 120, 121-132.

Miller, J.D. (1970) Audibility curve of the chinchilla. *J Acous Soc Am* 48, 513-523.

Murphy, W.J. and Themann, C.L. (1995) Hearing thresholds from auditory evoked potentials: Subcutaneous and implanted electrodes. *Assoc Res Otolaryngol* Abstr 548.

Nicotera, T., Henderson, D., Zheng, X.Y., Ding, D. and McFadden, S.L. (1999) Reactive oxygen species, necrosis and apoptosis in noise-exposed cochleas of chinchillas. *Assoc Res Otolaryngol* Abst.

Radi, R., Castro, L., Rodriguez, M., Cassina, A. and Tomson, L. (1997) Free radical damage to mitochondria. In: M.F. Beal, N. Howell, and I. Bodis-Wollner (Eds.), Mitochondria and Free Radicals in Neurodegenerative Disease, pp 57-89. New York: Wiley-Liss, Inc.

Romer, W., Oettel, M., Droescher, P. and Schwarz, S. (1997) Novel "scavestrogens" and their radical scavenging effects, iron-chelating, and total antioxidative activities: delta-8,9-dehydro derivatives of 17 α-estradiol and 17β-estradiol. *Steroids* 62, 304-310.

Ruiz-Larrea, M.G., Leal, A.M., Liza, M., Lacort, M. and de Groot, H. (1994) Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. *Steroids* 59, 383-511.

Seidman, M.D., Shivapuja, B.G. and Quirk, W. (1993) The protective effects of allopurinal and superoxide dismutase on noise-induced cochlear damage. *Otol Head Neck Surg* 106, 1052-1056.

Sharris, C. (1998). Personal communication regarding data compiled from the VA Administration. Washington. DC.

Snyder, D.L. and Salvi, R.J. (1994) A novel chinchilla restraint device. *Lab Anim* 23, 42-44.

Spoendlin, H. (1976) Anatomical changes following various noise exposures. In: D. Henderson, R.P. Hamernik, D.S. Dosanjh, and J.H. Mills (Eds.), *Effects of Noise on Hearing*. New York: Raven Press.

Yamane, H., Nakai, Y., Takayama, M., Iguchi, H., Nakagawa, T. and Kojima, A. (1995) Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. *Eur Arch Oto-Rhino-Laryngol* 252, 504-508.

Sex Differences in Auditory Sensitivity of Chinchillas Before and After Exposure to Impulse Noise

Sandra L. McFadden

Center for Hearing and Deafness, University of Buffalo, Buffalo, NY 14214-3001

Lynn W. Henselman

Office of the Inspector General, Department of Defense, 400 Army Navy Drive, Arlington, VA 22202

Xiang-Y. Zheng

Center for Hearing and Deafness, University of Buffalo, Buffalo, NY 14214-3001

9/14/98—"REVISED"

Corresponding Author:
Sandra L. McFadden, Ph.D.
Center for Hearing and Deafness
215 Parker Hall
University of Buffalo
Buffalo, NY 14214-3001
Voice: (716) 829-2001
Fax: (716) 829-2980
Email: mcfadden@acsu.buffalo.edu

Running head: Sex differences in chinchillas

ABSTRACT:

Objective: **To determine if chinchillas exhibit sex differences in (a) basic auditory sensitivity and (b) susceptibility to cochlear damage and hearing loss from high-level impulse noise.**

Design: **The auditory sensitivity of 73 chinchillas was assessed by measuring evoked potentials from electrodes implanted in the inferior colliculus (IC-EVPs) and cubic ($2f_1$-f_2) distortion product otoacoustic emissions (CDPs). A subgroup of 16 chinchillas were retested after exposure to simulated M16 rifle fire (150 dB pSPL impulse noise). Thresholds and post-exposure temporary and permanent threshold shifts (TTS and PTS, respectively) were compared as a function of sex and frequency using analysis of variance procedures. Cochleograms, showing the percent of hair cells missing as a function of location on the basilar membrane, were constructed to show inner hair cell (IHC) and outer hair cell (OHC) losses for each group.**

Results: **Female chinchillas had slightly lower high-frequency thresholds, and slightly higher low-frequency thresholds than male chinchillas, but similar IC-EVP and CDP amplitude functions. Significant sex differences were observed after exposure to high-level impulse noise. Overall, female chinchillas developed approximately 10 dB more high-frequency hearing loss, but approximately 5 dB less low-frequency hearing loss than males. Hair cell losses, particularly IHC losses, were substantially less for females as compared to males.**

Conclusions: **The results point to close similarities between chinchillas and humans with regard to sex/gender differences in basic auditory sensitivity prior to noise exposure, suggesting that the chinchilla may be a good model for exploring the anatomical and physiological bases of these differences. In addition, the results show significant sex differences in the physiological and anatomical response of the chinchilla cochlea to high-level noise. Similar differences in humans could have important implications with regard to military assignments and hearing conservation programs.**

INTRODUCTION

Noise-induced hearing loss (NIHL) is a major occupational hazard for military personnel due to the types and levels of noise encountered in training and combat situations (Dancer & Franke, 1986; Henselman, Henderson, Subramaniam, & Sallustio, 1994; Henselman, Henderson, Shadoan, Subramaniam, Saunders, & Ohlin, 1995). Damage to the cochlea can be caused by a variety of acoustic events, ranging from prolonged exposure to continuous noises that cause metabolic and biochemical changes in the cochlea, to relatively brief exposures to high-level impact and impulse noises such as gunfire, cannon fire and explosions, that can produce direct mechanical damage as well (Dancer & Frank, 1986; Henderson, Hamernik, & Sitler, 1974; Henderson, Spongr, Subramaniam, & Campo, 1994). A recognition of the serious consequences of NIHL led the U.S. Air Force to develop the first hearing conservation program (HCP) in 1948. The U.S. Navy and U.S. Army developed similar HCPs in 1955 and 1956, respectively (Henselman et al., 1995). Since their inception, military HCPs have served to increase awareness of the damaging effects of high-level noise exposure. They have also served to reduce the incidence and magnitude of NIHL in military personnel, primarily by mandating the use of personal protection devices (PPDs) such as sound-attenuating ear plugs or earmuffs in high-noise situations. However, NIHL remains a serious problem for military personnel who are exposed to loud noises during training and combat situations in which PPDs are either unavailable, impractical or dangerous to use, improperly fitted or worn, or inadequately designed to protect the ear from damage (Dancer et al., 1998).

As women become more fully integrated into a variety of military occupational specialties, many will be placed at risk for developing NIHL. It is critical, therefore, that we understand the specific relationship between noise exposure and hearing loss in women, so that appropriate measures for preventing NIHL can be developed and implemented.

Previous studies (Chung, Mason, Gannon, & Willson, 1983; Corso, 1963; Pearson, Morrell, Gordon-Salant, Brant, Metter, Klein, & Fozard, 1995; Ward, 1966) have reported small differences (generally less than 3 dB) between males and females in auditory sensitivity (i.e., thresholds for detecting pure tones under quiet listening conditions). In general, females tend to have slightly better pure-tone thresholds than males at frequencies above 1-2 kHz, while males may have slightly better thresholds below 1-2 kHz. Small, but consistent gender differences* have also been reported in susceptibility to temporary threshold shifts

* The term "gender differences" will be used to refer to male/female differences in humans, whereas the term "sex differences" will be used to refer to male/female differences in chinchillas and other non-human species.

(TTS) caused by exposure to continuous tones or noise (Axelsson & Lindgren, 1981; Dengerink, Dengerink, Swanson, Thompson, & Chermak, 1984; Petiot & Parrot, 1984; Ward, 1966). In general, experimental studies of TTS in humans have found that males exhibit more TTS than females from low-frequency exposures (below 2 kHz), whereas females exhibit more TTS than males from high-frequency exposures (above 2 kHz). In an early investigation of gender differences in susceptibility to TTS produced by high intensity tones and noise, Ward (1966) conducted 17 experiments with 24 male and 25 female adults. Females showed approximately 30% less TTS than males when the exposure frequency was below 1 kHz, but approximately 30% more TTS when the exposure frequency was above 2 kHz.

The above studies examined TTS rather than the more important issue of permanent threshold shift (PTS) because it is not ethical to intentionally induce PTS in human subjects. Most of what little we know about gender differences in PTS comes from retrospective studies of workers exposed to noise in industrial settings (Berger, Royster, & Thomas, 1978; Gallo & Glorig, 1964). Under these conditions, which typically involve exposure to low-frequency continuous noises, males tend to develop much more hearing loss than females. Both Berger et al. (1964) and Gallo and Glorig (1964) found approximately 20 dB more PTS in males than in females after nine years of industrial noise exposure. These results are consistent with the gender differences observed in Ward's (1966) studies of TTS. However, there are no comparable studies of gender differences in PTS produced by exposures to high-level impulse noises that are typically found in military environments. A finding of gender differences in susceptibility to NIHL could have important implications for military assignments and hearing conservation programs.

The present study was conducted to determine whether there are systematic differences between female and male chinchillas in (a) basic auditory function, as assessed by inferior colliculus evoked potentials (IC-EVPs) and cubic ($2f_1$-f_2) distortion product otoacoustic emissions (CDPs), and (b) their susceptibility to high-level impulse noise. Basic auditory function was assessed in a relatively large group of chinchillas (N=73). Susceptibility to impulse noise was examined in a subgroup of these animals (N=16). Findings from the chinchilla may shed light on gender differences in susceptibility to impact/impulse noise, and offer insights into the anatomical and physiological mechanisms contributing to documented gender differences in humans.

METHODS

All procedures described here were reviewed and approved by the University of Buffalo Animal Care and Use Committee, and conformed to federal guidelines for the humane treatment of laboratory animals.

Subjects

A total of 73 chinchillas (*Chinchilla laniger;* 37 female, 36 male) between 1 and 3 years of age were used. A subgroup of animals (8 female, 8 male) was exposed to impulse noise, and their thresholds were measured at various times after exposure (see below). The chinchilla was used for these studies because it (a) is relatively immune to middle ear infections and diseases that affect hearing (Clark, 1984); (b) has a relatively long life span (12-20 years) with minor age-related cochlear pathology and hearing loss prior to 8-10 years of age (Bohne, Gruner, & Harding, 1990; McFadden, Campo, Quaranta, & Henderson, 1997); and (c) reacts predictably to anesthesia and tolerates surgery well. Most importantly, the chinchilla has a range of hearing that is more similar to that of humans than most other laboratory animals, particularly in the low frequencies (Miller, 1970; Heffner & Heffner, 1991), which enhances its suitability as a model for studying NIHL (McFadden, Campo, Ding, & Quaranta, 1998). With regard to size, Clark (1984) states that female chinchillas tend to be larger than males. In a small group of our chinchillas (8 female, 8 male) for which reliable weights were available, weight differences were minor, but favored females. The average weight of females was 572.2 g (SD= 73.7), versus 563.9 g (SD=70.0) for males.

Surgical Preparation

Each animal was deeply anesthetized with an intramuscular (i.m.) injection of ketamine hydrochloride (60 mg/kg) and acepromazine (0.5 mg/kg). Chronic recording electrodes were implanted in the left and/or right inferior colliculus (IC), and in the rostral cranium (McFadden et al., 1997). Thirteen animals were implanted unilaterally; all others were implanted bilaterally. A small hole was drilled in the dorsal cranium overlying the IC, and a recording electrode mounted on a stereotaxic device was advanced through the IC while the surgeon monitored sound-evoked electrical activity on audio and video monitors. When the electrode had been advanced to a depth that produced clear, large amplitude EVPs, it was cemented to the skull with cyanoacrylic adhesive and dental cement. A short electrode was implanted in the rostral cranium to serve as the common lead for IC-EVP recording. Because the electrodes remain fixed in position, variability associated with changes in electrode placement between tests is eliminated. In addition, the signal-to-noise ratio is much better with implanted electrodes than with more conventional scalp electrodes, so that thresholds can be determined

with greater precision. IC-EVPs recorded from electrodes implanted in this manner yield thresholds that are very close to behavioral thresholds measured in the same animals (Henderson, Hamernik, Salvi, & Ahroon, 1983), and about 15-20 dB lower than threshold estimates obtained using subcutaneous electrodes in the same animals (Murphy & Themann, Reference Note 1). Following surgery, the animals recovered in a quiet animal colony for at least one week prior to testing.

Measures of Auditory Function

The auditory sensitivity of each animal was assessed by measuring IC-EVPs. CDPs were also obtained from most animals. All testing was conducted in a sound-attenuating booth (Industrial Acoustics Corp. 400) lined with sound-absorbing foam panels. The awake chinchilla was placed in a custom-designed tube (Snyder & Salvi, 1994) that held its head at a constant orientation within the calibrated sound field.

Stimuli for IC-EVP testing consisted of 10 ms tones (2 ms Blackman rise/fall ramp, alternating phase) at octave intervals from 0.5 to 16 kHz, presented at a rate of 20/sec. Stimuli were generated digitally (93 kHz sampling rate) by a 16 bit D/A converter on a digital signal processing (DSP) board (TMS320C25) in a personal computer (PC), and routed through computer-controlled attenuators and impedance matching transformers to a loudspeaker (Realistic 401197) located on the side of the test ear, at a distance of approximately 9 cm from the animal's pinna. The non-test ear was plugged with a foam insert earplug, providing approximately 20-40 dB attenuation in addition to the attenuation produced by the animal's head and body obstructing the propagation of sound to the opposite ear. Electrical activity from the IC electrode contralateral to the test ear was amplified (20,000 X), filtered (10-3000 Hz), and converted to digital signals (50 kHz sampling rate) by an A/D converter on a separate computer DSP board. Stimuli were presented in ascending order of frequency and intensity. Fifty or 100 trials were computer averaged at each stimulus level and the level was incremented in 5 dB steps. Figure 1 illustrates IC-EVP waveforms (raw data) obtained from a normal chinchilla.

Stored waveforms were analyzed visually to determine thresholds. Threshold (dB SPL re: 20 μPa) was defined as the mid-point between the level at which there was a clear deflection in the waveform and the next lower level at which there was none. For example, if there was a clear response at -5 dB SPL and none at -10 dB SPL, the threshold was recorded as -7.5 dB SPL (see Fig. 1).

CDP measurements were made using a system designed in our lab that utilizes three DSP boards housed in a PC, insert earphones (Etymotic ER-2), a low noise probe microphone (Etymotic ER-10B), and custom-built attenuators and

amplifiers. One DSP board processes microphone output while the other two generate digital signals (primary tones, f_1 and f_2). The primary tones were generated at a sampling rate of 93 kHz and output through 16 bit D/A converters. The microphone output was routed to a 16 bit A/D converter and digitized at a rate of 31 kHz. A Blackman windowing function was applied to the incoming data stream, and a partial discrete Fourier transform was computed. Frequency components corresponding to the two primary frequencies, the cubic distortion product ($2f_1$-f_2), and the noise floor (f_n=.7CDP) were computed. A calibration measurement preceded each input/output (I/O) function, in which the primary tones were presented at an attenuation of 20 dB and the output levels at the primary frequencies were measured and used as reference levels. Input/output functions were collected for primary tones (f_2 = 1.2, 2.4, 3.6, 4.8, 7.2, 9.6, and 12 kHz; f_2/f_1=1.2) from 0 to 70-80 dB SPL in 5 dB steps. CDP tests followed IC-EVP testing.

Noise Exposures and Acoustic Calibration

The impulse noise was a modified Friedlander wave (0.8 ms A-duration), with a time-amplitude profile simulating impulses created by 5.56 mm rounds fired from a U.S. Army M16A1 rifle (Danielson, Henderson, Gratton, Bianchi, & Salvi, 1991). The digital signal was converted to analog by a D/A converter on a DSP board, attenuated (HP 350D), amplified (NAD 2200), and routed in parallel to two compression drivers (JBL 2446) coupled to sound delivery tubes (5 cm dia X 20 cm). The ends of the sound delivery tubes were cut at 45° angles to broaden the range of the tube's resonance (Danielson et al., 1991). The drivers faced each other, with the sound delivery tubes separated by 10 cm. Acoustic foam wedges surrounded the drivers to minimize reverberation. An animal was placed in a restraint tube in the 10 cm space between the opposing sound tubes, and 50 pairs of impulses (100 total) were delivered simultaneously to both ears. Impulses in each pair were spaced 50 ms apart, and there was a 1000 ms interval between the onset of each pair (Henselman et al., 1994). The duration of the exposure was therefore less than one minute for each animal.

All exposures were conducted in a 1.8 m X 2.0 m sound booth (Acoustic Systems), where animals were exposed individually. A 1/8" microphone (Bruel & Kjaer Model 4138) was used for acoustic calibration of the impulse noise. The voltage corresponding to a 114 dB tone produced by a pistonphone coupled to the microphone was determined, and used to calculate the desired voltage for a 150 dB peak SPL signal. The attenuation of a manual attenuator (Hewlett Packard 350D) was adjusted to produce the desired signal voltage.

Test Schedule after Noise Exposure

IC-EVPs and CDPs were measured from impulse noise-exposed animals at 15 min, 24 h, and 5 days post-exposure in order to monitor TTS, and after 25-35 days recovery from exposure in order to determine PTS. Prior to exposure, each animal was tested three times, and the average of the three measurements was used as the stable baseline estimate of sensitivity. Threshold shifts of each animal were calculated by subtracting mean pre-exposure IC-EVP thresholds from post-exposure thresholds. After 25-35 days recovery from high-level exposure, IC-EVPs and CDPs were measured on three separate occasions and averaged in order to calculate PTS at each frequency.

Cochlear Histology

At the end of testing, chinchillas were deeply anesthetized with sodium pentobarbital (Somlethal, 100 mg/kg i.p.) and decapitated. The cochleas were quickly removed and perfused through the oval window with a succinate dehydrogenase (SDH) staining solution (2.5 ml, 0.2 M sodium succinate, 2.5 ml, 0.2 M phosphate buffer, pH 7.6, and 5 ml, 0.1% tetranitro blue tetrazolium). Cochleas were then incubated in the SDH staining solution for 45 min at 37 °C, post-fixed with 10% formalin, and stored in fixative. Stained cochleas were dissected from the apex to the base, mounted in sections in glycerin on microscope slides, coverslipped, and examined using light microscopy (400X magnification). Percent hair cells missing was referenced to our lab standards based on average hair cell counts from 9 cochleas of young (<1 yr old), healthy chinchillas.

Data Analyses

Data were obtained from both ears of 37 animals (19 male, 18 female) and from a single ear of 36 animals, so that the final sample for data analysis consisted of 110 ears (55 male, 55 female). Noise-exposure data were obtained from 28 ears (15 male, 13 female). Data analyses were geared toward answering the following questions: (1) Are there significant sex differences in auditory sensitivity, IC-EVP amplitudes, or CDP I/O functions? (2) Are there sex differences in TTS and/or PTS caused by exposure to simulated M16 rifle fire? Analyses of variance (ANOVAs) were used to assess differences between means. The dependent variables were IC-EVP thresholds and IC-EVP threshold shifts at various times after noise exposure. Independent variables were Sex (a between-subjects factor), Frequency and Time of Assessment (within-subjects factors). Significant main effects and interactions involving Sex were analyzed further using one-way ANOVAs or independent Student t-tests. Within a group, changes as a function of time or frequency were assessed using paired t-tests. Mean IC-

EVP and CDP amplitude functions for females and males were compared by calculating the 95% confidence interval for the difference between the means. All statistical tests were evaluated using a 0.05 criterion of significance.

RESULTS

Basic Auditory Sensitivity of Female and Male Chinchillas

IC-EVP Thresholds. Thresholds of female and male chinchillas are shown in Figure 2. As a group, male chinchillas have slightly lower thresholds than females at frequencies below 2 kHz, while female chinchillas have slightly lower thresholds than males at frequencies above 2 kHz. The differences are generally small, but consistent.

A two-way mixed ANOVA, with Sex as a between-subjects factor and Frequency as a within-subjects factor, revealed a significant Sex X Frequency interaction, $F(5,540) = 7.58$, $p < 0.001$. Follow-up analyses indicated that mean threshold at 16 kHz was significantly higher for males than for females (16.15 ± 4.8 dB vs. 11.64 ± 5.7 dB), $F(1,108) = 20.24$, $p < 0.0001$. Thresholds at frequencies below 16 kHz were not significantly different between the two sexes.

IC-EVP and CDP Amplitude Functions. Mean IC-EVP input/output functions at 0.5, 1, 2, 4, 8 and 16 kHz are shown in Figure 3. The thin lines represent means for female chinchillas, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent the means for male chinchillas. It is apparent from Figure 3 that there were no significant sex differences in mean I/O functions despite slight differences in IC-EVP thresholds (see Fig. 2).

Similarly, there were no meaningful differences between male and female chinchillas in their CDP I/O functions (Fig. 4). The thin lines in Figure 4 represent means for females, and the hatched regions surrounding them represent the 95% confidence intervals. The thick lines represent means for males. The CDP frequency is indicated above each panel. CDP I/O functions were very similar for males and females, with thresholds around 20-30 dB SPL at all frequencies, and amplitudes increasing monotonically over the entire range of input levels. Overall, the results indicate that there are no meaningful sex differences in amplitudes of either IC-EVPs or CDPs prior to noise exposure, despite small differences in thresholds (Fig. 2).

Sex Differences in Hearing Loss from Simulated M16 Rifle Fire

Pre-exposure Thresholds. Pre-exposure IC-EVP thresholds for the subset of animals exposed to noise are shown in Figure 5. Although females exhibited slightly lower thresholds than males at several frequencies, particularly at 8 and

16 kHz, a two-way (Sex X Frequency) mixed ANOVA did not detect significant differences between the sexes.

Post-exposure TS. Mean IC-EVP threshold shifts measured at 15 min, 24 hr, and 5 days after exposure to 150 dB peak SPL impulse noise are shown in Figure 6. When tested 15 min after the high-level exposure, both females and males exhibited significant threshold elevations (47-68 dB) at all frequencies (Fig. 6A). Males showed approximately 5-6 dB more TS than females at 0.5 and 1 kHz, whereas females showed approximately 6 dB more TS than males at 8 and 16 kHz. A two-way mixed (Sex X Frequency) ANOVA indicated that there was a significant Sex X Frequency interaction, $F(5,130)=2.1$, $p = 0.05$, but no main effect of Sex. Follow-up analyses indicated that TS increased progressively from 0.5 kHz to 8 kHz for females. Differences between successive frequencies were significant from 0.5 kHz to 4 kHz; TS was equivalent at 4 and 8 kHz, then declined significantly between 8 and 16 kHz (all p values < 0.01). In contrast, males showed a much flatter pattern of TS, with statistically equivalent TS at 1, 2, 4, and 8 kHz.

Relatively little threshold recovery occurred between 15 min and 24 hr post-exposure. Females showed TS decreases of 0-7 dB, while males showed TS decreases of 0-9 dB. Both females and males showed the greatest recovery (5-9 dB) at 4 and 8 kHz. As shown in Figure 6B, TS ranged from approximately 47 dB at 0.5 kHz to 61 dB at 8 kHz. Females exhibited approximately 9 dB more TS than males at 8 and 16 kHz, and approximately 5 dB less TS than males at 1 kHz. A two-way ANOVA yielded a significant Sex X Frequency interaction, $F(5,130)=2.4$, $p = 0.044$. Whereas females had statistically equivalent TS at 2, 4, 8 and 16 kHz and significantly less TS at 0.5 and 1 kHz, males had equivalent TS at 1, 2, 4 and 8 kHz, and significantly less TS at 0.5 and 16 kHz than at intermediate frequencies.

Significant recovery occurred between 1 and 5 days after exposure, with TS decreasing by 9-19 dB. Females and males showed equivalent TS at frequencies ≤ 4 kHz, whereas females exhibited approximately 9 dB and 11 dB more TS than males at 8 and 16 kHz, respectively (Fig. 6C). However, the two-way (Sex X Frequency) ANOVA did not reveal any significant differences between the sexes at this time.

Mean thresholds improved by 4-13 dB between 5 and 30 days post-exposure, when permanent hearing loss was assessed (Fig. 7). High-level exposure produced significant PTS at all frequencies for both females and males (paired t-tests; all p values < 0.001). PTS ranged from 23-43 dB, with females showing 2-7 dB less PTS than males at low frequencies (0.5-2 kHz), and approximately 9 dB more PTS at 8 and 16 kHz. A significant Sex X Frequency interaction was obtained, $F(5,130) = 3.10$, $p = 0.011$. Follow-up analyses indicated that both

males and females developed progressively and significantly greater PTS from 0.5 to 2 kHz. However, PTS peaked at 2 kHz for males, and declined significantly at higher frequencies. Females, in contrast, had significantly greater PTS at 8 and 16 kHz than at lower frequencies.

CDP amplitude data are generally consistent with the IC-EVP data. Before noise exposure, CDP I/O functions were similar for males and females, as shown in Figure 8. After exposure, however, CDP amplitudes were significantly depressed (Fig. 9). There was a trend for males to have lower amplitude CDPs than females at low frequencies (where males had greater PTS), but higher CDP amplitudes at high frequencies (where males had less PTS).

Hair Cell Losses. Sixteen cochleas (8 female, 8 male) were examined for hair cell losses. As shown in Figure 10, OHC loss (left panel) exceeded IHC loss (right panel), with OHC losses ranging from 70-100% in the basal half of the cochlea for both sexes. Males sustained substantially greater IHC and OHC losses than females. IHC losses for males peaked in the 2-3 kHz region of the cochlea, with an average loss of approximately 80%. In contrast, average IHC losses for the females did not exceed 30% in any region of the cochlea. OHC losses of females were approximately 20% less than OHC losses of males in the cochlear regions (>1 kHz) where OHC loss occurred.

DISCUSSION

The results indicate that female and male chinchillas differ slightly in their basic auditory sensitivity, with females tending to have lower thresholds at high frequencies and higher thresholds at low frequencies. More importantly, the results point to a fundamental sex difference in the response of the chinchilla cochlea to high-level impulse noise. Female chinchillas sustained more high-frequency hearing loss, less low-frequency hearing loss, and less hair cell loss than males. The reasons for the sex differences observed both before and after noise exposure cannot be determined from this study. However, because the differences were observed in chinchillas, they cannot be attributed to differences in noise exposure history, recreational activities, dietary factors, or other extraneous variables that complicate interpretation of gender differences in humans (Henderson, Subramaniam, & Boettcher, 1993). Therefore, the data from the chinchilla may be particularly useful in interpreting findings from previous studies with humans.

Small but consistent gender differences in auditory sensitivity have been well documented in humans (e.g., Chung et al., 1983; Corso, 1963; Pearson et al., 1995; Ward, 1966). In general, females tend to have slightly lower pure-tone thresholds than males at frequencies above 1-2 kHz, while males may have

slightly lower thresholds below 1-2 kHz. Chung et al. (1983) analyzed data from more than 50,000 people and found that the average difference between males and females in hearing sensitivity was 2-3.5 dB for test frequencies above 2 kHz, and less than 1 dB for frequencies at or below 2 kHz. Ward (1966) found that thresholds of young adult females were up to 6 dB better than thresholds of young adult males at frequencies above 2.8 kHz. Although differences in auditory sensitivity have sometimes been attributed to gender-related differences in noise exposure history, the current data from chinchillas argue for inherent anatomical and/or physiological differences between the sexes. Recently, Pearson et al. (1995) reported the results of the Baltimore Longitudinal Study of Aging, which tracked thresholds of 681 men and 416 women in low-noise occupations who were screened for otological disorders and noise-induced hearing loss. Their results provide further evidence of small gender differences in thresholds while ruling out occupational noise exposure as the cause for poorer thresholds in men. Women had significantly better thresholds than men at all frequencies above 1 kHz, while men had better thresholds at 0.5 kHz, and men and women did not differ at 1 kHz.

Sex/gender differences in both basic sensitivity and in susceptibility to NIHL could arise from differences in the acoustical properties of the outer and middle ears. In a recent study, Hellstrom (1995b) showed a significant relationship between the sound transfer function (STF) of the external ear, ear canal dimensions, and hearing levels in male and female subjects. Females tended to have ear canals that were shorter and smaller in volume than males, resulting in an average STF that was shifted toward higher frequencies. Gender differences in the STF of the external and middle ears would be expected to influence susceptibility to NIHL as well as basic auditory sensitivity (Hellstrom, 1995a,b; Saunders and Tilney, 1982; Tonndorf, 1976).

We are not aware of any published studies of sex differences in ear canal characteristics in non-human species. Consequently, the possibility that the sex differences observed in the present study are due to systematic differences in STFs cannot be ruled out. However, several factors suggest that the STF is not the sole basis for sex/gender differences in chinchillas or humans. First, data presented by Saunders and Tilney (1982) show that the chinchilla ear canal STF is a sharply peaked function, with gain increasing from approximately 5 dB SPL at 4.8 kHz to 23 dB at 10 kHz, then dropping to 5 dB around 14 kHz. This STF contrasts with the human ear canal STF, which has a resonant peak between 2 and 4 kHz (Hellstrom, 1995b), yet the pattern of sex/gender differences for chinchillas and humans are quite similar. Hellstrom himself noted that certain aspects of his data were difficult to account for in terms of STF. In particular, there is no obvious

reason why subjects with elevated STFs at 4 kHz tended to have lower thresholds at 2 kHz than subjects with elevated STFs at 2 kHz.

A second point to consider is that there are numerous gender differences that are not easily accounted for by the STF. Gender differences have been observed in (a) the upper limit for perceiving binaural beats (Tobias, 1965), with women having a significantly lower cut-off frequency than men (600 versus 800 Hz), (b) the incidence of spontaneous otoacoustic emissions (SOAEs), with women exhibiting them significantly more often than men (Bell, 1992; Bilger, Matthies, Hammel, & DeMorest, 1990; Whitehead, Baker, & Wilson, 1989), and (c) auditory brainstem responses, with women having shorter central conduction times, even after differences in head size are taken into account (Edwards, Squires, Buchwald, & Tanguay, 1983; Patterson, Michalewski, Thompson, Bowman, & Litzelman, 1981; Trune, Mitchell, & Phillips, 1978). Gender differences such as these suggest that factors other than simple acoustics may be involved. Third, studies have reported that hearing sensitivity (Baker & Weiler, 1977; Cox, 1980; Davis & Ahroon, 1982; Miller & Gould, 1966; Swanson & Dengerink, 1988), SOAEs (Bell, 1992; Penner, 1995), auditory brainstem responses (Elkind-Hirsch, Stoner, Stach, & Jerger, 1992), and susceptibility to TTS (Davis & Ahroon, 1982; Dengerink, Dengerink, Swqanson, Thompson, & Chermak, 1984; Petiot & Parrot, 1984) can all fluctuate in monthly cycles in women, or differ between normally-cycling women and women taking oral contraceptives. The above considerations suggest that factors other than (or in addition to) the STF may be responsible for sex/gender differences in basic auditory sensitivity and susceptibility to NIHL. Future studies using the chinchilla may help determine the relative importance of anatomical and physiological factors in sex/gender differences in auditory sensitivity and susceptibility to NIHL.

FIGURE LEGENDS

Figure 1. Typical IC-EVP waveforms obtained from a normal chinchilla. Stimulus frequency was 8 kHz. Threshold is –7.5 dB SPL.

Figure 2. Pre-exposure thresholds of female (solid circles) and male (open triangles) chinchillas. Differences between females and males were statistically significant at 16 kHz only. Bars represent standard errors of the means.

Figure 3. Pre-exposure IC-EVP input/output functions for female (thin line) and male (thick lines) chinchillas. Hatched regions represent the 95% confidence interval for the difference between means.

Figure 4. Pre-exposure CDP input/output functions for female (thin line) and male (thick lines) chinchillas. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

Figure 5. Pre-exposure thresholds of the female (solid circles) and male (open triangles) chinchillas that were subsequently exposed to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

Figure 6. Threshold shifts of female (solid circles) and male (open triangles) chinchillas at 15 m, 24 h, and 5 days after exposure to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

Figure 7. Permanent threshold shifts of female (solid circles) and male (open triangles) chinchillas, measured 30 days after exposure to 150 dB pSPL impulse noise. Bars represent standard errors of the means.

Figure 8. CDP input/output functions for female (thin line) and male (thick lines) chinchillas prior to noise exposure. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

Figure 9. CDP input/output functions for female (thin line) and male (thick lines) chinchillas 30 days after noise exposure. Hatched regions represent the 95% confidence interval for the difference between means. The parameter above each panel indicates the CDP frequency ($2f_1$-f_2).

Figure 10. Mean hair cell losses after impulse noise exposure. Left panel, OHC loss; right panel, IHC loss.

ACKNOWLEDGMENTS

This research was supported by Grant DAMD17-96-1-6330 from the U.S. Army Medical Research & Materiel Command and the Department of Defense Women's Health Research Program to SLM. Portions of the data were collected as part of Lieutenant Colonel Henselman's Ph.D. dissertation at University of Buffalo. The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. The authors are grateful to Marty Howard for assistance in data collection and noise exposures.

REFERENCES

Axelsson, A., & Lindgren, F. (1981). Pop music and hearing. *Ear and Hearing, 2*, 64-69.

Baker, M. A., & Weiler, E. M. (1977). Sex of listener and hormonal correlates of auditory thresholds. *British Journal of Audiology, 11*, 65-68.

Bell, A. (1992). Circadian and menstrual rhythms in frequency variations of spontaneous otoacoustic emissions from human ears. *Hearing Research, 58*, 91-100.

Berger, E. H., Royster, L. H., & Thomas, W. G. (1978). Presumed noise-induced permanent threshold shift resulting from exposure to an A-weighted Leq of 89 dB. *Journal of the Acoustical Society of America, 64*, 192-197.

Bilger, R. C., Matthies, M. L., Hammel, D. R., & DeMorest, M. E. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech and Hearing Research, 33*, 418-432.

Bohne, B. A., Gruner, M. M., & Harding, G. W. (1990). Morphological correlates of aging in the chinchilla cochlea. *Hearing Research, 48*, 79-91.

Chung, D. Y., Mason, K., Gannon, R. P., & Willson, G. N. (1983). The ear effect as a function of age and hearing loss. *Journal of the Acoustical Society of America, 73*, 1277-1282.

Clark, J. D. (1984). Biology and diseases of other rodents. In: J.G. Fox, B.J. Cohen & F.M. Lowe (Eds.), *Laboratory Animal Medicine*. New York: Academic Press Inc.

Corso, J. F. (1963). Age and sex differences in pure tone thresholds: survey of hearing levels from 18 to 65 years. *Archives of Otolaryngology, 77*, 385-405.

Cox, J. R. (1980). Hormonal influence on auditory function. *Ear & Hearing, 1*, 219-222.

Dancer, A., Buck, K., Parmentier, G., & Hamery, P. (1998). The specific problems of noise in military life. *Scandinavian Audiology, 27*, 123-130.

Dancer, A., & Franke, R. (1986). Effects of weapon noise on hearing. In: R.J. Salvi, D. Henderson, R.P. Hamernik, & V. Colletti (Eds.), *Basic and Applied Aspects of Noise-Induced Hearing Loss.* New York: Plenum Press.

Danielson, R., Henderson, D., Gratton, M. A., Bianchi, L., & Salvi, R. (1991). The importance of "temporal pattern" in traumatic impulse noise exposures. *Journal of the Acoustical Society of America, 90*, 209-218.

Davis, M. J., & Ahroon, W.A. (1982). Fluctuations in susceptibility to noise-induced temporary threshold shift as influenced by the menstrual cycle. *Journal of Audiology Research, 22*, 173-187.

Dengerink, J. E., Dengerink, H. A., Swanson, S., Thompson, P., & Chermak, G. D. (1984). Gender and oral contraceptive effects on temporary auditory effects of noise. *Audiology, 23*, 411-425.

Edwards, R. M., Squires, N. K., Buchwald, J. S., & Tanguay, P. E. (1983). Central transmission time differences in the auditory brainstem response as a function of sex, age, and ear of stimulation. *International Journal of Neuroscience, 18*, 59-66.

Elkind-Hirsch, K.E., Stoner, W.R., Stach, B.A., & Jerger, J.F. (1992). Estrogen influences auditory brainstem responses during the normal menstrual cycle. *Hearing Research; 60,* 143-148.

Gallo, R., & Glorig, A. (1964). Permanent threshold shift changes produced by noise exposure and aging. *Journal of Industrial Hygiene, 25*, 237-245.

Heffner, R. S., & Heffner, H. E. (1991). Behavioral hearing range of the chinchilla. *Hearing Research, 52*, 13-16.

Hellstrom, P.-A. (1995a). Individual differences in peripheral sound transfer function: Relation to NIHL. In: A. Axelsson et al. (Eds.), *Scientific Basis of Noise-Induced Hearing Loss*. New York: Thieme, pp. 110-116.

Hellstrom, P.-A. (1995b). The relationship between sound transfer functions and hearing levels. *Hearing Research, 88*, 54-60.

Henderson, D., Spongr, V. P., Subramaniam, M., & Campo, P. (1994). Anatomical effects of impact noise. *Hearing Research, 76*, 101-117.

Henderson, D., Subramaniam, M., & Boettcher, F. A. (1993). Individual susceptibility to noise-induced hearing loss: An old topic revisited. *Ear and Hearing, 14*, 152-168.

Henderson, D., Hamernik, R. P., & Sitler, R. W. (1974). Audiometric and histological correlates of exposure to 1-ms noise impulses in the chinchilla. *Journal of the Acoustical Society of America, 56*, 1210-1221.

Henderson, D., Hamernik, R. P., Salvi, R. J., & Ahroon, W. H. (1983). Comparison of auditory-evoked potentials and behavioral thresholds in normal and noise-exposed chinchillas. *Audiology, 22*, 172-180.

Henselman, L. W., Henderson, D., Shadoan, J., Subramaniam, M., Saunders, S., & Ohlin, D. (1995). Effects of noise exposure, race, and years of service on hearing in U.S. Army soldiers. *Ear and Hearing, 16*, 382-391.

Henselman, L. W., Henderson, D., Subramaniam, M., & Sallustio, V. (1994). The effect of 'conditioning' exposures on hearing loss from impulse noise. *Hearing Research, 78*, 1-10.

McFadden, S. L., Campo, P., Quaranta, N., & Henderson, D. (1997). Age-related decline of auditory function in the chinchilla (*Chinchilla laniger*). *Hearing Research, 111*, 114-126.

McFadden, S. L., Campo, P., Ding, D. L., & Quaranta, N. (1998). Effects of low-frequency noise on evoked potentials and cochlear anatomy in young and aged chinchillas. *Hearing Research, 117*, 81-96.

Miller, J. D. (1970). Audibility curve of the chinchilla. *Journal of the Acoustical Society of America, 48*, 513-523.

Miller, M.H., & Gould, W.J. (1967). Fluctuating sensorineural hearing impairment associated with the menstrual cycle. *Journal of Audiology Research, 7*, 373-385.

Patterson, J. V., Michalewski, H. J., Thompson, L. W., Bowman, T. E., & Litzelman, D. K. (1981). Age and sex differences in the human auditory brainstem response. *Journal of Gerontology, 36*, 455-462.

Pearson, J. D., Morrell, C. H., Gordon-Salant, S., Brant, L. J., Metter, E. J., Klein, L. L., & Fozard, J. L. (1995). Gender differences in a longitudinal study of age-associated hearing loss. *Journal of the Acoustical Society of America, 97*, 1196-1205.

Penner, M.J. (1995). Frequency variation of spontaneous otoacoustic emissions during a naturally occurring menstrual cycle amenorrhea and oral contraceptive: A brief report. *Ear & Hearing, 16*, 428-432.

Petiot, J.-C., & Parrot, J. E. (1984). Effects of the ovarian and contraceptive cycles on absolute thresholds auditory fatigue and recovery from temporary threshold shifts at 4 and 6 kHz. *Audiology, 23*, 581-598.

Saunders, J. C., & Tilney, L. G. (1982). Species differences in susceptibility to noise exposure. In: R.P. Hamernik, D. Henderson, & R. Salvi (Eds.), New Perspectives on Noise-Induced Hearing Loss. New York: Raven Press, pp. 229-248..

Snyder, D. L., & Salvi, R. J. (1994). A novel chinchilla restraint device. *Laboratory Animal, 23*, 42-44.

Swanson, S.J., & Dengerink, H.A. (1988). Changes in pure-tone thresholds and temporary threshold shifts as a function of menstrual cycle and oral contraceptives. *Journal of Speech & Hearing Research, 31*, 569-574.

Tobias, J. V. (1965). Consistency of sex differences in binaural beat perception. *International Audiology, 4*, 179-182.

Tonndorf, J. (1976). Relationship between the transmission characteristics of the conductive system and noise-induced hearing loss. In: D. Henderson, R.P. Hamernik, D.S. Dosanjh, & J.H. Mills (Eds.), *Effects of Noise on Hearing*. New York: Raven Press, pp. 159-177.

Trune, D. R., Mitchell, C., & Phillips, D. S. (1978). The relative importance of head size, gender and age on the auditory brainstem response. *Hearing Research, 32*, 165-174.

Ward, D. W. (1966). Temporary threshold shift in males and females. *Journal of the Acoustical Society of America, 40*, 478-485.

Whitehead, M. L., Baker, R. J., & Wilson, J. P. (1989). The bilateral symmetry and sex asymmetry of spontaneous otoacoustic emission (SOAE) incidence in human ears. *British Journal of Audiology, 23*, 149.

REFERENCE NOTES

1. Murphy, W. J., & Themann, C. L. (1995). Hearing thresholds from auditory evoked potentials: Subcutaneous and implanted electrodes. *Association for Research in Otolaryngology, Abstract 548.*

Differences Between Female and Male Chinchillas in Susceptibility to Noise-Induced Hearing Loss

S.L. McFadden, X.Y. Zheng, D.L. Ding, and D. Henderson

Center for Hearing and Deafness, University of Buffalo, Buffalo, NY 14214

Small gender differences in auditory sensitivity have been well documented in humans. In addition, experimental studies of temporary threshold shifts (TS) and retrospective studies of hearing loss in industrial workers suggest that males are more susceptible to TS from low-frequency exposures, whereas females are more susceptible to TS from high-frequency exposures. Whether these differences are due to differences in noise exposure history, diet, and recreational activities or to inherent anatomic and/or physiological factors has not been determined. Furthermore, there is no information regarding gender differences in susceptibility to impulse noise. We conducted 6 separate experiments in which evoked potential thresholds were obtained from female and male chinchillas before and after exposure to noise. Each experiment utilized a different noise exposure condition: (1) 4 kHz octave band noise (OBN) at 105 dB SPL for 4 h; (2) impulse noise (simulated M16 rifle fire) at 150 dB pSPL with (a) a single driver positioned in front of the animal and (b) two drivers facing each ear and firing simultaneously; (3) 0.5 kHz OBN at 90-95 dB SPL for 5 days, followed by impulse noise; (4) noise simulating an Army Blackhawk helicopter at (a) 90 dB SPL for 5 days, followed by impulse noise, and (b) 112 dB SPL for 5 days, followed by impulse noise. The results show that prior to exposure, female chinchillas tend to have higher low-frequency thresholds and lower high-frequency thresholds than males. The differences are generally small (less than 5 dB SPL), but consistent across experiments. After exposure, males showed more low-frequency hearing loss (up to 15 dB) than females in all 6 experiments. Females showed more high-frequency hearing loss (up to 30 dB) than males in 4 experiments. Cochleograms generally showed greater hair cell losses in males than in females. Overall, the results suggest that there are sex differences in basic auditory sensitivity and in the physiological and anatomical response of the chinchilla cochlea to damaging levels of noise. Future studies with the chinchilla may be useful for understanding the nature of gender differences in humans.

Supported by Grant DAMD17-96-1-6330 to SLM.