2007

EFFECTS OF SOURCE, GENDER, AND AGE AT SOUND EXPOSURE ON HEARING IN C57BL/6J MICE

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THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTERS OF SCIENCE

Graduate Studies Office

2007

SIGNATURE APPROVAL FORM

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ABSTRACT

EFFECTS OF SOURCE, GENDER, AND AGE AT SOUND EXPOSURE ON HEARING IN C57BL/6J MICE

By

Alyssia Joni Rogers

The C57BL/6J mouse is commonly used as a model of human presbycusis. While human males are more susceptible to presbycusis compared to human females, the role of sex in the hearing loss of C57BL/6J mice is controversial. Similarly, subject source—laboratory vs. commercial vendor—has been linked to differentially accelerated patterns of hearing loss. Finally, the interaction between disease and timing of noise exposure has been explored in a variety of studies, with sometimes disparate results. Subjects in the current study included males and females, bred at NMU or obtained from the Jackson Laboratory. Thresholds were measured at 2 and 6.5 months of age. One group of mice was sound exposed (11.2 kHz ½ octave band of noise, 101 dB noise spectrum level, for 76 minutes) at two months of age, while a second group was exposed at 6.5 months. Thresholds in all subjects were re-assessed at 7.5 months of age. Female mice had lower but not significantly different thresholds than males. Data indicated that subject source had no influence on auditory brainstem response thresholds. Age-at-exposure data supported the hypothesis that acoustic over stimulation is more detrimental to the young auditory nervous system that is genetically predisposed to early-onset deafness compared to mature hearing structures.

DEDICATION

This thesis is dedicated to my great friends who love to play and listen to live music, and whom I hope will read this and begin protecting their fragile ear structures in order to continue fully enjoying music late into life.

ACKNOWLEDGMENTS

The writer would like to thank committee chair, Cynthia Prosen, Ph.D for her never-ending patience, effort, support, and motherly kindness. Thanks also to committee members Sheila Burns, Ph.D and Jackie Bird, Ph.D for their advice and suggestions made to improve this project. Additionally, the writer would like to thank undergraduate Kathleen Curran for her help in the collection of ABR thresholds, and Mike Strahan for his assistance. This research was supported by the National Institutes of Health (R15 DCO4405).

This thesis follows the format prescribed by the *APA Style Manual* and the Department of Psychology.

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ABBREVIATIONS

ABR: Auditory brainstem response

AHL: Age-related hearing loss

ANOVA: Analysis of Variance

dB: Decibel

IHC: Inner hair cells

kHz: Kilohertz

NIHL: Noise-induced hearing loss

NSL: Noise spectrum level

OBN: Octave band noise

OHC: Outer hair cells

PTS: Permanent threshold shift

SPL: Sound pressure level

TTS: Temporary threshold shift

INTRODUCTION

Adult-onset progressive *sensorineural hearing loss* is known as *presbycusis*. This disease causes hearing loss as a function of the aging process (Willems, 2000). Sensorineural hearing loss is a result of damaged sensory cells (hair cells) in the *cochlea* and is usually permanent. Presbycusis is the most common neurodegenerative disease (Ohlemiller, Wright, & Heidbreder, 2000). Deficits in hearing from this condition are a result of both genetic and environmental factors (Willems, 2000). Reports are contradictory concerning how presbycusis interacts with certain variables over the lifetime of an individual. There is no clear-cut procedure for the prevention or treatment of this disease. Furthermore, experimental studies in animals show intersubject variability regarding presbycusis and its effects on hearing (Prosen, Dore, & May, 2003). This type of hearing loss is a common and serious problem in modern society influenced by the combined effects of the environment, aging, and heredity.

Hearing is a complex sense involving sensitivity and *frequency* selectivity of the ear and interpretation of sound, along with the ability to understand speech. Mild losses in hearing may go unnoticed and even moderate losses may not be a problem for those with excellent perceptual abilities and good coping skills. Extensive research has attempted to determine the pathological changes characteristic of presbycusis, but the exact biological and physiological mechanisms of the disease and its course remain unknown. Hence, electrophysiological and anatomical research in an animal model of presbycusis can help further characterize the disease, and may ultimately improve treatment.

GLOSSARY OF TERMS

Ahl Gene: A recessive gene that maps to Chromosome ten which is found in mice exhibiting age-related hearing loss. The Ahl gene is also implicated in the susceptibility to noise-induced hearing loss.

Apex: The inner tip of the spiral-shaped cochlea; the opposite end from the base (Moore, 1998).

Attenuate: The reduction in amplitude and intensity of a signal (Turkington, 2004).

Auditory Brainstem Response (ABR): A physiological measure of the electrical activity in the brainstem that can determine how well certain portions of the auditory system in the brain, particularly the eighth cranial nerve, respond to a presented stimulus. A brief tone causes a small variation in the electrical potentials recorded from the scalp. Clicks or tones are presented; the stimulus is repeated up to 1000 times and averaged, while eliminating background electrical activity, thus developing auditory thresholds (Turkington, 2004).

Base: The end of the cochlea closer to the oval window and stapes (Moore, 1998).

Basilar Membrane: A membrane inside the cochlea that vibrates in response to sound and whose vibrations lead to activity in the auditory pathways (Moore, 1998). This membrane divides the cochlea lengthwise into two compartments. On one side of the membrane is the perilymph fluid of the scala tympani; on the other side is the organ of Corti. As sound vibrations disturb the perilymph fluid, they are transferred through the basilar membrane to the organ of Corti and on to the hair cells inside (Turkington, 2004).

C57BL/6J mouse: C57BL/6J mice experience a rapid progression of presbycusis-like hearing deficits and thus provide a convenient animal model for evaluating behavioral, physiological and anatomical correlates of the disorder (Henry, 2002).

CBA/CaJ mouse: Strain of mouse said to possess 'the Gold Standard of hearing.'

Chronological age: Age measured by the time (years and months).

Cochlea: Spiral portion of the bony labyrinth of the inner ear that surrounds the organ of hearing (Martini, 1999).

Deafness: People with shifts in threshold larger than 75 dB (NIDCD, 2004).

Decibel (dB): One tenth of a bel and is equal to ten times the logarithm (base 10) of the ratio of two intensities, or 20 times the logarithm of the ratio of two amplitudes or pressures (Moore, 1998).

Eighth cranial nerve: Also called the auditory, hearing, or cochlear nerve, it is really two separate nerves, one responsible for transmitting sound and the other with sending balance information to the brain from the inner ear. The two nerves intertwine and pass through the bony canal leading from the inner ear to the brain. This nerve is the only connection between the hair cells of the cochlea and the cochlear nucleus of the brainstem and is essential for good hearing. It is about 25 mm long and contains about 32,000 nerve fibers (Turkington, 2004).

External Auditory Canal: Also known as the ear canal or meatus. This tube extends from the floor of the external ear inward to the eardrum (tympanic membrane) (Turkington, 2004).

Fibrocytes: These cells shape the curve of the cochlea's lateral wall and buffer mechanical forces generated by sound vibrations (Delprat et al., 2005).

Frequency: For a sine wave, the frequency is the number of periods occurring in one second. The unit is cycles per second, or hertz (Hz) (Moore, 1998).

Functional age: Categorizes loss by the nature of the loss rather than the age of onset (Prosen et al., 2003).

Functional age 1: Determined by hearing changes at higher frequencies in background noise. These deficits were noted at chronological ages 23 and 26.5 weeks (Prosen et al., 2003).

Functional ages 2: Defined by high frequency threshold elevations in conditions of quiet, noted at the ages 22-31 weeks (Prosen et al., 2003).

Functional age 3: Characterized by deficits of low frequency hearing with background noise, noted at the ages 27-51 weeks (Prosen et al., 2003).

Functional age 4: Characterized by deficits of low frequency hearing without background noise, noted at the ages 27-51 weeks (Prosen et al., 2003).

Inner hair cells (IHCs): Specialized cells lying between the basilar membrane and the tectorial membrane.They are transducers that convert mechanical vibration into electrical neural responses (action potentials or spikes) in the auditory nerve (Moore 1998).

Kilohertz (kHz): A unit of frequency equal to 1000 Hz (Yost, 1999).

Loudness: The subjective impression of the magnitude of a sound. It is defined as the attribute of an auditory sensation in terms of which sounds may be ordered on a scale from quiet to loud (Moore, 1998).

Noise: Unwanted sound (Moore, 1998).

Noise Spectrum Level (NSL): The level of sound in decibels measured in a 1-Hz wide band. It is often used to characterize sounds with continuous spectra such as noise. A white noise has a long-term average spectrum level that is independent of frequency (Moore, 1998).

Octave band noise (1/3): An octave is the interval between two discrete frequencies having a frequency ratio of two. For instance, frequencies of 25 Hz and 50 Hz are said to be separated by one octave. A one-third octave band is defined as a frequency band whose upper band-edge frequency (f_2) is the cube root of two times the lower band frequency (f_1). (Yost, 1999).

Organ of Corti: A complex structure lying between the basilar membrane and the tectorial membrane, playing an important role in the active mechanisms of the cochlea (Moore, 1998).

Outer hair cells (OHCs): Specialized cells lying between the basilar membrane and the tectorial membrane. They play an important role in the active mechanisms of the cochlea (Moore, 1998).

Perilymph fluid: A fluid, almost identical to spinal fluid, which is contained in the canals of the cochlea (Turkington, 2004).

Permanent threshold shift (PTS): Shifts in auditory thresholds that are not reversible.

Presbycusis: The hearing loss that is associated with aging (Moore, 1998).

Scala tympani: One of the two outer chambers of the cochlea. It lies on the opposite side of the basilar membrane from the organ of Corti (Moore, 1998).

Scala vestibuli: One of the two outer chambers of the cochlea. It lies on the same side as the basilar membrane from the organ of Corti (Moore, 1998).

Sensorineural hearing loss: A general term used to describe hearing loss caused by damage to the cochlea and/or the auditory nerve and higher levels of the auditory pathway (Moore, 1998).

Sociocusis: Hearing loss from non-occupational noise exposure. In addition to actual damage to the auditory mechanism, research has revealed that noise may contribute to loss of sleep, tension, headaches, reduced vision, sexual impotence, heart disease and

mental illness, which in turn contribute to more complex psychological and social problems (www.sfu.ca/sonic-studio/handbook/Sociocusis.html).

Sound Pressure Level (SPL): The level of sound in decibels relative to an internationally defined reference level as measured by a standard sound level meter with a microphone (Moore, 1998).

Spiral or cochlear ganglion: Contains the cell bodies of the sensory neurons that monitor the receptors in the cochlear duct (Martini, 1999).

S*tereocilia:* Responsible for the transduction of mechanical sound energy into electrical signals (Yost, 2000)

Tectorial Membrane: A gelatinous structure lying above the stereocilia of the inner and outer hair cells (Moore, 1998).

Temporary Threshold Shifts (TTS): Commonly experienced following exposure to sound levels over 70 to 75 dB, and are reversible as a function of time (Takagi et al., 1988).

Tinnitus: The perception of a sound in the absence of an external sound applied to the ear (Moore, 1998).

Tone: A sound wave capable of evoking an auditory sensation having pitch (Moore, 1998).

Tympanic membrane: The membrane that separates the external acoustic meatus from the middle ear; membrane whose vibrations are transferred to the auditory ossicles and ultimately to the oval window (Martini, 1999).

Characteristics of Presbycusis

Presbycusis involves the premature loss of hearing that occurs gradually in many individuals as a result of the aging process. About 25 to 35 percent of adults between the ages of 65 and 75 suffer from this type of hearing loss (Marcincuk and Roland, 2002; National Institute on Deafness and Other Communication Disorders [NIDCD], 2004). It is estimated that 40 to 50 percent of people age 75 and older have some type of hearing loss (NIDCD, 2004).

Presbycusis begins with a loss of hearing at higher frequencies, progressing to lower frequencies as a function of age (Yost, 2000). This disease can begin as early as 30 years of age, with effects only becoming noticeable at, on average, age 55 for men and age 64 for women (Goldstein, 1999). Thus, there is a time when presbycusis is sub-clinical (Cohn, 2002). Lack of awareness pertaining to the onset of this disease may cause further problems in terms of exposure to hearing-damaging agents. The degree to which hearing sensitivity has been affected by presbycusis can be measured by observing shifts in auditory thresholds, since as presbycusis advances, hearing thresholds progressively worsen (Goldstein, 1999). The lower limit of audibility is defined as 0 dB (*decibel*), but the upper limit is not as clearly defined. A more relevant limit is one at which the ear will be physically harmed causing a hearing disability, which depends on the time and type of exposure to intense *noise*. An increase in auditory thresholds of 25 dB generally requires adaptive listening strategies such as hearing aids, which amplify surrounding environmental stimuli. By comparison, the term *deafness* is applied to people with shifts in threshold larger than 75 dB (NIDCD, 2004).

The human ear can detect sounds in the range .02 kHz (*kilohertz*) to 20 kHz. This upper limit tends to decrease with age, such that most adults cannot hear above 16 kHz. Loss of hearing from presbycusis initially affects sounds of frequencies 8, 6, and 4 kHz. Hearing loss then progresses to frequencies of 2-3 kHz, which includes those frequencies used in human speech (Cohn, 2002). Speech discrimination is often worsened by the addition of background noise, and speech sounds often seem less clear. High-pitched sounds such as "s" and "th" are often difficult to distinguish (NIDCD, 2004). Furthermore, a lower male voice may be easier to hear and interpret than a (higher frequency) female voice. *Tinnitus*—a ringing in one or both ears—may also occur with presbycusis (NIDCD, 2004).

Hearing disorders are exacerbated by background noise. Consequently, people with presbycusis often cannot communicate successfully in everyday situations (Gilad & Glorig, 1979). Hearing loss may eventually contribute to the social isolation of elderly people by restricting their use of the telephone, causing them to abandon social opportunities such as concerts and social gatherings, and ultimately augment their sense of disability.

As the median age of American society increases, presbycusis will become a more prevalent disease. The deficits mentioned above are a result of the deterioration the auditory system experiences as the body ages. To understand the pathologies of hearing loss due to presbycusis, a brief summary of normal auditory function is needed.

Anatomy of the Auditory System

The auditory system is comprised of three parts—the outer, middle, and inner ear (Figure 1). The inner ear regulates two sensory systems: the vestibular system—designed for spatial orientation and equilibrium—and the auditory system, which is responsible for the hearing process.

Among the parts of the outer ear is the pinna, which funnels sound into the *external auditory canal* or ear canal, and provides protection to the delicate structures enclosed within the middle and inner part of the ear (Yost, 2000). The ear canal provides directional sensitivity to the ear by blocking or facilitating the passage of sound to the *tympanic membrane*, or eardrum, a thin semi-transparent membrane that separates the external ear from the middle ear (Martini, Timmons, & Tallitsch, 2006).

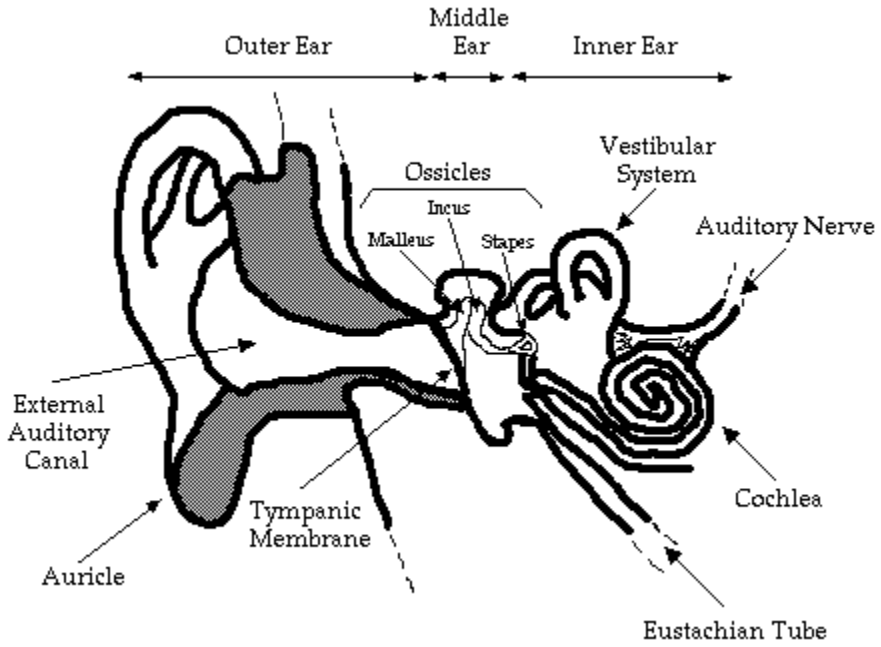

Figure 1. The outer, middle, and inner parts of the ear (http://www.utdallas.edu/~thib/rehabinfo/te.htm). Photograph copyright by Linda M. Thibodeau, PhD. Permission granted.

The middle ear consists of the tympanic membrane, the tympanic cavity (an air-filled space), the middle ear bones or ossicles (malleus, incus, and stapes) which are suspended in the middle ear cavity, and the oval window (Figure 1) (Martini et al., 2006).

The inner ear, a fluid-filled bony structure, contains an intricate system of chambers known as the membranous labyrinth. A shell of dense bone (the bony labyrinth) surrounds and protects the membranous labyrinth. Between the bony and membranous labyrinths flows *perilymph fluid* (Martini, 2006). The bony labyrinth houses three primary cavities: the vestibule, the semicircular canals, and the fluid-filled cochlea (Yost, 2000). The cochlea (Figure 2) is the primary hearing organ. Its name is derived from the Greek word meaning snail and it is a snail-shaped organ, turning in humans 2 5/8 times, as seen in Figure 2. The cochlea has a *base* and an *apex* and contains 3 lymph-filled channels running the length of the coil. At the base of the cochlea, the *scala vestibuli*

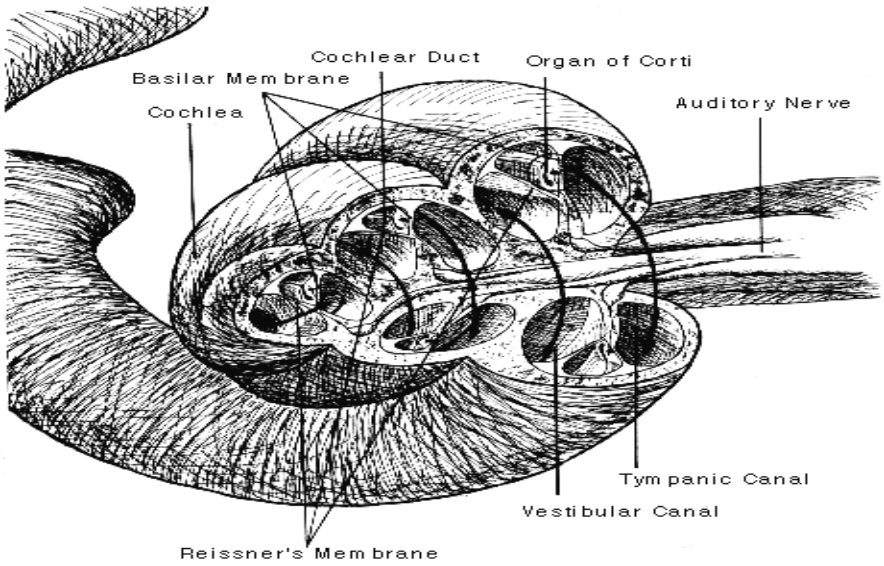

Figure 2. A sectional view of the cochlea.
From Truax, Barry. (1999). Cambridge Street Publishing.
www.sfu.ca/~truax. Photograph copyright by Barry Truax, Professor.
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terminates at the oval window where the footplate of the stapes sits (Martini, 2006). The *scala tympani* ends at the round window. The cochlea's function is to convert sound waves into neural impulses. These impulses are then carried through the *Eighth cranial nerve* to various specialized cells in the central auditory system and then to the auditory cortex (Yost, 2000).

The cochlea contains the *organ of Corti*, the sensory transduction system of the ear, which houses the receptor cells, or hair cells. The surface of these sensory epithelial cells have bundles of s*tereocilia* which are fundamental to the hearing process. The organ of Corti extends the length of the cochlea sandwiched between the *tectorial membrane* (which is firmly attached to the inner wall of the cochlear duct) and the *basilar membrane* (Figure 2) (Yost, 2000).

Hair cells are divided into two types—inner and outer hair cells. There are one row of *inner hair cells* (IHCs) and three rows of *outer hair cells* (OHCs). On the inner side of the organ of Corti are the IHCs, while the OHCs are found on the outer side of the organ of Corti (Yost, 2000). Humans possess approximately 3,500 IHCs and 12,000 OHCs. These stereocilia of graded height protrude from the cells' surfaces (Figure 3). Hair cells are made of stiff protein strands called actin. Inner hair cells contain straight rows of stereocilia, while outer hair cells have "w" or "v" -shaped rows of stereocilia. Stereocilia are responsible for the transduction of mechanical sound energy into electrical signals (Yost, 2000). Outer hair cells expand and contract when stimulated by external sound stimuli. It is these cells that contribute to both hearing sensitivity and frequency discrimination (Yost, 2000).

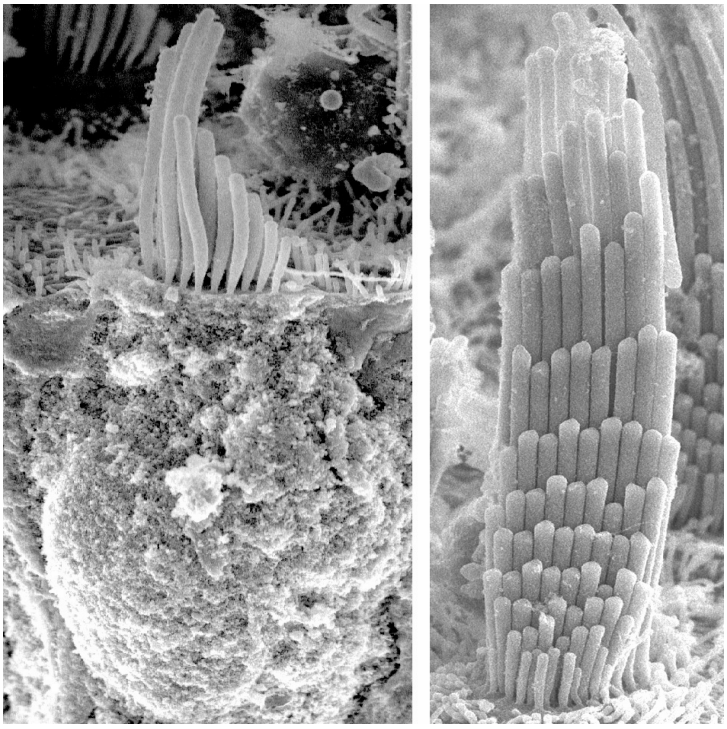

Figure 3. An electron micrograph of a sensory hair cell (right) and a hair bundle (left) (http://www.keele.ac.uk/depts/co/auditory).
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Physiology of the Auditory System

The physiological process of hearing involves all parts of the ear mentioned above, and each part of the ear has a different role in transmitting sound from the environment, to where it is perceived by the brain. When sound stimuli from the external environment are funneled through the ear canal to the eardrum, the eardrum converts these sounds into vibrations. As sound moves through the ear canal, intensity of high frequency sounds increase by up to 20 dB. In adults, the frequencies heard best are at 2.5 kHz, while the peak intensities for children are at higher frequencies (Martini, 2006).

Transmitting sound from the environment to the inner ear is done by way of the ossicular chain (malleus, incus, and stapes). These three bones act as levers that transfer

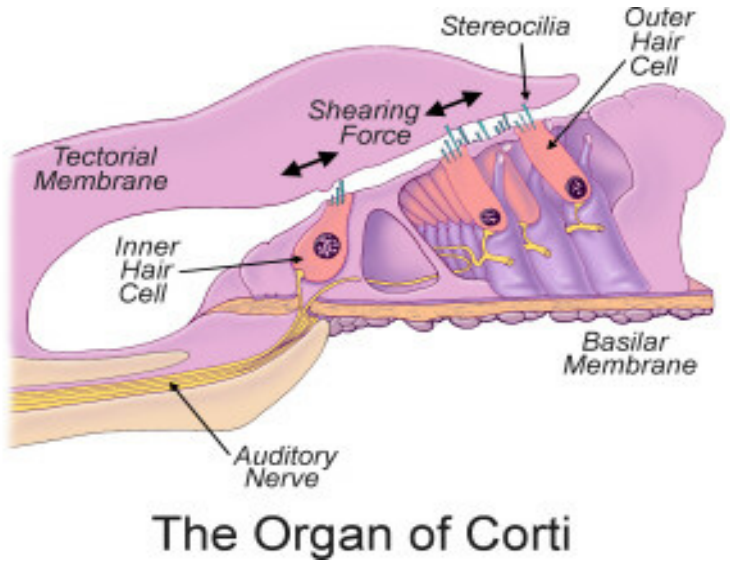

Figure 4. The organ of Corti located within the cochlea (http://www.bcm.edu/oto/research/cochlea/hearing/JOHCFIG3.jpg). Copyright by John S. Oghalai, MD. Permission granted.

sound vibrations to the perilymph fluid where movement of the ossicles applies pressure within the inner ear (Martini, 2006).

The fluid movement within the inner ear to the cochlea causes the tectorial membrane and stereocilia to be stimulated, resulting in a shearing motion between the two structures, and the stereocilia bend at the base (see Figure 4) (Yost, 2000). The displacement of fluid within the inner ear also results in a traveling wave-like motion of mechanical energy beginning at the base of the cochlea and continuing toward the apex. This movement allows mechanical energy to be transduced into electrochemical activity by the organ of Corti (Brown, 1999). Stereocilia of the inner hair cells within the organ of Corti are bent in response to the cochlear vibrations, and the neural response generated inside the IHCs is transmitted to the auditory nerve fibers, sending a signal up the auditory nerve bundle of the eighth cranial nerve to the brainstem. The OHCs, on the other hand, feed energy back into the cochlea increasing its' sensitivity, and serving as an active amplifier of incoming sound (Yost, 2000). Hair cells transduce stimuli in

microseconds, in contrast to the tens to hundreds of milliseconds required in the transduction of photoreceptors and olfactory neurons (Hudspeth & Konishi, 2000)

The traveling wave peaks at different parts of the basilar membrane, depending on the frequency of the sound wave. Higher frequency sounds stimulate the wide basal end of the cochlea, while lower frequency stimuli stimulate the narrow apical end of the cochlea (Goldstein, 1999). Each increment of the approximately 30-mm-long basilar membrane is tuned to a particular frequency. In fact, a trained musician can discriminate changes in frequency with the precision of 0.1% (Hudspeth & Konishi, 2000). The amount of movement at a given location on the basilar membrane depends on the amount of force applied to the oval window. This relationship provides a mechanism for detecting the intensity, or volume, of the sound. Because the rest of the cochlea is sheathed in bone, pressure applied to the oval window can only be relieved at the round window; thus, sound is absorbed here (Martini, 2006).

The bending of the stereocilia caused by the traveling wave along the basilar membrane is part of their normal function. Very high intensity sounds can produce hearing losses by breaking the stereocilia off the surface of the hair cells (Martini, 2006). Thus, in order to function properly for long periods of time, the stereocilia need to be strong and resilient (Yost, 2000).

Factors Causing Damage to Stereocilia Leading to Loss of Hearing

A number of factors can damage hair cells. For example, aging causes the hair cells to lose their flexibility, which increases vulnerability to damage and eventual cellular death. Another type of stress that may be a factor in age-related hearing loss

(AHL) is oxidant stress. An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed 'oxidative stress'. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions (Sies, 1997). Also resulting in hair cell death and the consequent loss of hearing is the bending, and in some cases flattening, of the stereocilia due to loud, excessive external auditory stimulation. Additionally, hair cells are not replaced; therefore their number declines throughout life (Hudspeth & Konishi, 2000). The stereocilia on the hair cells located at the base of the cochlea are more vulnerable to trauma than those at the apex. The process of damage by any of these mechanisms can be accelerated by exposure to excessive noise (Mills, Gilbert, & Adkins, 1979).

Destruction of the stereocilia as a result of age, disease, genetic, and environmental factors diminishes the ability of these hair cells to mediate hearing. When damaged, hair cells cannot transmit sounds to the brain as efficiently as when the cells are intact (Yost, 2000). Among the factors causing stereocilia damage are oxygen radicals — atoms of oxygen with an unpaired electron. Oxidative stress, the accumulation of free radical mediated tissue damage, is a core mechanism of aging. Aerobic metabolism results in the creation of hydrogen peroxide and reactive oxygen species which can result in cellular damage, including damage to cell membranes, DNA, and other large molecules, and initiation of apoptosis (cell death). Free radicals are normally detoxified by a variety of enzymes and free radical scavengers in the body (Staecker and Van De Water, 1999). Oxygen radicals are also found in cells exposed to alcohol, drugs, pollutants, and even mental stress (Pobojewski, 2006). It has been suggested that there

are increased levels of oxidative stress in the aging *C57BL/6J* mouse cochlea. This strain of mouse exhibits age-related hearing loss (Staecker and Van De Water, 1999).

Certain chemical agents, known as ototoxins, can also harm the stereocilia (International Organization for Standardization (ISO), 1990). Ototoxicity is defined as the tendency of certain agents to cause functional impairment and cellular degeneration of the inner ear and the eighth cranial nerve. Over 200 agents have been reported as ototoxic, including antibiotics belonging to the "mycin" family and salicylates such as aspirin. Certain chemotherapy drugs and anti-inflammatory drugs are known to be ototoxic. Agents that cause ototoxicity commonly found in the workplace include solvents such as benzene, toluene, butanol, and trichloroethylene. Additionally, arsenic, lead, cobalt, mercury, and lithium are considered ototoxic. Some agents are also synergistic with over-exposure to loud noise. That is, in circumstances where neither the agent nor the noise exposure alone produces a hearing loss, the combination of the two does. Examples of these agents include carbon disulfide and carbon monoxide (ISO, 1990). Many diseases can damage the stereocilia, including rubella, meningitis, diabetes, renal disease, rheumatoid arthritis, and Meniere's disease (ISO, 1990).

Destruction of hair cells also occurs by way of deterioration and eventual cell death in conjunction with the natural aging process because hair cells are particularly prone to damage at the basal end of the cochlea (Mills et al., 1979). Detection and discrimination of high frequency stimuli are often affected first. This is because sounds of all frequencies enter the organ of Corti through the base of the cochlea, impacting these cells first (ISO, 1990).

Unfortunately, like most types of human cells, hair cells and their stereocilia do not regenerate. Fish, birds, and other cold-blooded species can replace damaged hair cells. Additionally, supporting cells can serve as hair-cell precursors in these animals (Hudspeth & Konishi, 2000). Recent work by Raphael and his colleagues offers the exciting possibility of mammalian hair cell regeneration providing the opportunity for the restoration of hearing (Raphael, Kobayashi, Dootz, Beyer, Dolan, & Burmeister, 2001). However, at present, damage to mammalian cochlear receptor cells is irreversible.

Due to ethical, financial, and practical issues, humans are not ideal research subjects. How variables discussed above (e.g., age and the environment) affect presbycusis and the auditory system must be studied in non-human animals. The use of animals as models in auditory research allows more precise control over genetic and environmental variables than is possible in humans. Animal models also allow the study of age-related effects over shorter periods of time.

C57BL/6J: A Model for Presbycusis

The relationship between cochlear damage and hearing loss is the subject of both basic and clinical research questions. Mice are inexpensive and easily maintainable subjects (Prosen et al., 2003). Also, their size makes it simple to house them in a small space, maximizing the potential number of subjects in the study. Finally, mice are a model of age-related hearing loss due to the varying hearing abilities of genetically different strains.

The *CBA/CaJ* mouse strain is said to possess the 'gold standard' of hearing function (Erway, Shiau, Davis, & Krieg, 1996). These mice are used to study normal

hearing as well as serve as control subjects in studies on abnormal hearing function. Another strain of mice, C57BL/6J, is genetically predisposed to age-related hearing loss (Erway et al., 1996). The C57BL/6J strain is of mild temperament and works well in behavioral studies (Prosen et al., 2003). Most importantly, this strain is an excellent model of early adult-onset progressive sensorineural hearing loss, or presbycusis. These mice progressively lose their hearing over a relatively short time frame (Henry and Chole, 1980). Mice have a life span of approximately 2 to 2 ½ years, and C57BL/6J mice exhibit a transition from normal hearing to profound deafness during the first 18 months of life (Li and Borg, 1991). Since audiological research with humans is hindered due to the lateness in onset of the pathology, a mouse model allows for the testing of hypotheses over a relatively short time period (Johnson, Erway, Cook, Willot, & Zheng, 1997).

Both CBA/CaJ and C57BL/6J mice exhibit greatest hearing sensitivity to stimuli at 16 kHz (Zheng, Johnson & Erway, 1999). But while the CBA/CaJ strain shows only minimal elevations in hearing thresholds until 24 months of age, with a loss reaching 45 dB by 30 months, the C57BL/6J strain reaches this same degree of hearing loss at just 8 months of age (Ingham, Thornton, Comis, & Withington, 1998). *The auditory brainstem response* (ABR) is an electrophysiologic measure of hearing. In young CBA/CaJ mice, ABR to *tones* at the frequencies of 8, 16, 32 kHz, and to click (includes all frequencies) stimuli were 24 dB *sound pressure level* (SPL), 15 dB SPL, 39 dB SPL, and 36 dB SPL, respectively (Zheng et al., 1999). These low thresholds for CBA/CaJ mice show they do not demonstrate any measurable amount of hearing loss at one year of age (Zheng et al., 1999). In comparison, thresholds of C57BL/6J were normal at 33 weeks, but at two years of age were 60 dB above normal means. Thresholds in C57BL/6J mice at two years of

age to tones at 8, 16, 32 kHz and click stimuli were 84 dB SPL, 78 dB SPL, 105 dB SPL, and 106 dB SPL. These thresholds demonstrate that C57BL/6J mice show measurable hearing loss as a function of age (Zheng et al., 1999).

Unfortunately, interanimal variability exists in the functional and structural changes among exposed animals (Wang, Hirose, & Liberman, 2002). For example, Mizuta, Nozawa, Morita, & Hoshino (1993) reported considerable variation in degeneration of outer hair cells when compared to C57BL/6J mice described by Hulcrantz and Li (1995).

Because genetic hearing loss of C57/BL/6J mice is variable, Prosen et al. (2003) described the concept of *functional age* rather than *chronological age* to more fully describe the hearing loss seen in behaviorally-trained C57BL/6J mice. Functional age categorizes by the nature of the loss rather than the age of the loss. Comparisons of auditory thresholds from a group of C57BL/6J mice suggest that the hearing loss of this strain occurred in particular stages. The earliest hearing impairments were detected when behavioral testing was conducted in conditions of background noise, suggesting that humans with presbycusis may first notice a loss of hearing in noisy environments. Subsequent loss involved threshold elevations in conditions of quiet. In both instances, the hearing impairments proceeded from high to low frequencies. The median ages for 16 and 8 kHz threshold shifts were 27 and 33 weeks, respectively. The thresholds of both groups were higher at 16 kHz than at 8 kHz (Prosen et al., 2003). The time course of hearing loss often began with a gradual threshold elevation and ended with an abrupt and severe loss (Prosen et al., 2003).

Prosen et al. (2003) described four stages of hearing loss in C57BL/6J mice. *Functional age 1* was determined by hearing changes at higher frequencies in background noise. These deficits were noted at chronological ages 23 and 26.5 weeks. *Functional age 2* was defined by high frequency threshold elevations in conditions of quiet, noted at ages 22-31 weeks. *Functional ages 3and 4* were characterized by deficits of low frequency hearing with and without background noise, respectively. Criteria for functional ages 3 and 4 were reached in subjects for 8 kHz tones at ages 27-51 weeks. These data showed the progression of hearing loss from high-to-low frequencies, and from listening environments of noise to quiet, as has been described by humans with presbycusis.

High frequency threshold shifts are due to degeneration of cochlear hair cells which normally spreads to the *spiral (or cochlear) ganglion* cells (Li and Borg, 1991), which receives input from inner hair cells mediating the initial stages of auditory perception (Fritzsch, Farinas, & Reichardt, 1997). Low frequency threshold shifts occurred as early as 27 weeks and as late as 54 weeks in some subjects. Rather than being a consequence of hair cell loss in the apex of the cochlea, threshold shifts at lower frequencies are correlated with degeneration of cells in the lateral wall of the cochlea (Prosen et al., 2003), which is made up of collagen bundles produced by *fibrocytes*. These cells shape the curve of the cochlea's lateral wall and buffer mechanical constraints caused by sound vibrations (Delprat, Ruel, Guitton, Hamard, Lenoir, Pujol, Puel, Brabet, & Hamel, 2005).

The behavioral and anatomical results of Prosen et al. (2003) suggest that the C57BL/6J strain is an adequate model of presbycusis in humans. These data also

emphasize that problems arise when using only age-based studies in mice to characterize the pattern of loss seen in presbycusis.

Variability of Characterization of Hearing Loss

Several factors have been proposed to contribute to the variability in hearing sensitivity found in C57BL/6J mice. The current study investigated three of these potential factors. The first variable tested was subject source, i.e., from where the subject originated. It has been suggested that laboratory-born subjects have lower and more uniform thresholds compared to subjects obtained from a commercial vendor. This factor must be considered because of potential noise trauma experienced during transportation, especially by air travel. Thus, comparing C57BL/6J thresholds of laboratory-raised mice to thresholds of C57BL/6J mice obtained from a commercial vendor—and subsequently transported to the laboratory—would show whether transportation is a factor that increases the variability between subjects, and additionally causes loss of hearing.

Sex was a second factor evaluated as a source of variability in C57BL/6J mice. Human males are more susceptible to presbycusis than human females. While human females are said to hear better than human males, the discrepancy between the two is correlated with genetic and environmental factors (Gates, Couropmitree, & Myers, 1999). It is unknown how environmental and genetic factors account for gender differences in humans. In the mouse model for presbycusis, the opposite effect has been seen. Female mice showed characteristics of presbycusis earlier than male mice (Henry, 2002). The role of gender in the hearing loss of C57BL/6J mice is controversial (Goldstein, 1999).

Because of the discrepancies seen between genders, this issue was further examined in this report.

Finally, the interaction between presbycusis onset and timing of acoustic over-exposure is a relationship that has been explored in several studies, with conflicting or inconsistent results (Bohne and Clark, 1982; Coleman, 1976; Davis, Cheever, Krieg, & Erway, 1999; Dayal, and Bhattacharyya, 1986; Erway et al., 1996; Falk, Cook, Haseman, & Sanders, 1974; Hu, Henderson, & Nicotera, 2002; Jimenez, Stagner, Martin, & Lonsbury-Martin, 2001; Kujawa, Liberman, Brinsko, Rosowski, Tempel, 2003; Li, Hultcrantz, & Borg, 1993; Maison and Liberman, 2000; Miller, Dolan, Raphael, & Altschuler, 1998; Mikaelian, Warfield, & Norris, 1974; Ohlemiller et al., 2000; Pujol, 1992; Shone, Altschuler, Miller, & Nuttall, 1991). Experimental results from most noise-exposure studies were gained by testing post-exposure thresholds almost directly following exposure to noise; e.g., days or weeks post-exposure. This does not allow the evaluation of long-term post-exposure effects in mice insulted by noise when young. Measuring post-exposure thresholds for animals exposed to noise at young or old age, at the same chronological age post-exposure would allow an evaluation of the interaction between the effects of time at noise exposure and the aging process.

1. Hearing loss as a function of age

The stability of auditory thresholds of CBA/CaJ subjects is much different compared to the rapid onset of hearing loss observed in C57BL/6J mice. Many studies have validated the use of C57BL/6J mice in age-related hearing loss research (Davis et al., 1999; Erway et al., 1996; Hultcrantz and Li, 1995; Mizuta et al., 1993; Ohlemiller et

al., 2000). Not only do C57BL/6J mice exhibit presbycusis-like loss with elevated hearing thresholds as a function of age, the anatomical pattern of hair cell loss seen in these mice is similar to the hair-cell loss seen in humans with presbycusis (Mizuta et al., 1993). Hearing loss in C57BL/6J mice begins in the first two months of age, when the mouse is considered a young adult. At three months of age, cochlear degeneration begins in the basal portion of the organ of Corti, with OHC loss of 80% and IHC of approximately 55% (Mikaelian et al., 1974). However, few hair cells are missing at 3 months of age in the apical or innermost part of the C57BL/6J cochlea (Spongr, Flood, Frisina, & Salvi, 1997). Amount of hearing loss in this strain reaches moderate levels at six months of age. According to Li and Borg (1991), the aging processes in the auditory system of C57BL/6J mice show dramatic changes between 6 and 7 months, and 9 and 10 months.

The pattern of loss seen in this strain of mouse is due to progressive degeneration of the organ of Corti and other cochlear structures (Li and Borg, 1991). By month eight, OHC and IHC losses are seen throughout the entire cochlea (Spongr et al., 1997). In the extreme basal portion, the OHC loss is 100%, whereas the apical region shows only 15-20% damage to hair cells. For IHCs, the basal cochlea shows 87% degeneration, while the middle two-thirds of the cochlea suffers from approximately 15-20% IHC loss (Spongr et al., 1997). By the end of one year, the organ of Corti is severely damaged, disappearing from the basal turn, and appearing only in degenerative form in the apical section of the cochlea. Hearing loss becomes extreme by 15 months of age (Mikaelian et al., 1974).

Because there are large individual differences between reported thresholds of subjects in the midst of a hearing loss, it is difficult to determine the extent of presbycusis on an individual subject solely by age (Li and Borg, 1991; Hulcrantz and Li, 1995; Prosen et al., 2003). For example, the amount and pattern of hair cell damage caused by the same noise exposure or other insult can vary greatly between individuals (Yost, 2000). Thus, other factors must be considered to determine the etiology of hearing loss.

2. The Effects of Source on Hearing Loss

The C57BL/6J inbred mouse strain is the most widely used strain of mice in auditory research (Johnson et al., 1997). Thus, it is imperative to be certain that when laboratories receive these mice to use for auditory research, the mice do not have a pre-existing hearing loss, because of travel-related sound exposure. As a corollary, human infants are exposed to excessive noise from ambulance and air transport. The highest sound levels were recorded during air transport. Average sound levels for all modes of transportation exceeded the recommended levels for neonatal intensive care. The maximum sound levels recorded were greater than 80 dB, and greater than 120 dB in the total frequency range, i.e., sounds that are beyond the human hearing range (Buckland, Austin, Jackson, & Inder, 2003). Whether subject transportation causes hearing loss before the experiment even begins is important to insure precise research concerning the auditory system. The current study is the only known report that took this idea into consideration.

3. The Effects of Sex on Hearing Loss

Another factor that may differentially contribute to hearing loss is sex. Human males are more affected by presbycusis compared to human females. More than 50% of males over the age of 65 sustain loss of hearing (Johnson et al., 1997), compared to 37 % of human females over the age of 65 (Garstecki and Erler, 2001). Alternatively, older women have been reported to have poorer low-frequency hearing sensitivity than do men (NIDCD, 2004). Loss of estrogen as women age may affect hearing sensitivity at certain frequency levels (Cooper, 1994). The fact that women have more sensitive hearing at frequencies above 1 kHz while men have more sensitive hearing at lower frequencies is known as "gender reversal" (Pearson, Morrell, Gordon-Sarant, Brant, Metter, Klein, & Fozard, 1995).

Studies concerning gender differences in hearing in humans suggest that men are more prone to hearing loss because of the environmental effects of noise exposure during work and pleasure-related activities. In a clinical study using males and females of approximately 70 years old, men had worse hearing than women (Gates, 1999). Less hearing loss in females than males in industrialized societies compared to non-industrial societies supports the idea that men are on average exposed to more damaging noise and thus suffer from more noise-induced hearing loss (NIHL) (Kryter, 1985). Kryter (1985) compared data from humans with no history of exposure to intense noise in the workplace to patients with presbycusis, and also to *sociocusis*—non-work related noise-induced hearing loss. Evidence that industrialized societies leave males more susceptible to hearing loss is supported by lack of sex differences in the Mabaan tribe (Kryter, 1985). The fact that gender differences are not seen in some non-industrialized societies suggests

that if the confound of noise exposure was eliminated there would be no gender differences in humans with presbycusis.

In contrast to the Kryter (1983) data, Pearson et al. (1995) conducted a longitudinal study with 681 men and 416 women, to rule out environmental sources of noise as confounding factors. Only human subjects exposed to low levels of noise were used in this experiment. It was found that sensitivity in hearing declined more than twice as fast in men than in women, with changes in men's hearing detectable by 30 years of age. Thus, the assumption that men lose their hearing at a faster pace than women due solely to exposures to noise may not be valid (Pearson et al., 1995).

The question of whether one gender is biologically more susceptible to hearing loss following exposure to noise has not been completely resolved in human studies. Many studies have been conducted in mice to evaluate whether one gender is a more appropriate model to use in presbycusis research (Henry, 2002; Ehret 1979; Hunter and Willott, 1987). Female C57BL/6J mice bred and maintained in a laboratory controlled for noise suffered more from presbycusis than male mice in the same situation (Henry, 2002). Alternately, Hunter & Willott (1987) collected ABR thresholds using equal size groups of male and female C57BL/6J mice of various age groups (1, 2-3, 4-5, 6-7, 12-13 months). Results of this study showed no significant difference between male and female mice in any age group. Thus, contradicting research regarding sex discrepancies shows that more can be done using a mouse model to study differences in humans.

4. Hearing Loss as a Function of Acoustic Overexposure

One environmental factor impacting the health of hair cells is acoustic over-stimulation. High intensities of noise have been associated with many health effects in adults, including hearing loss and high blood pressure (Joint Committee on Infant Hearing, 1999). The *loudness* of sound is measured in decibels, a logarithmic scale (Committee on Environmental Health, 1997). When loud and excessive sound impacts the eardrums, the stapes rapidly pushes fluid within the inner ear, with the potential to cause damage to the hair cells (Arakaki, 2000). Constant exposure to intense auditory stimuli weakens the hair cells, until the stereocilia are unable to return to their normal position. After a recovery period of several days to one month following intense exposure to noise, the histological appearance of the ear is considerably different from that seen acutely. The recovery period allows irreversibly damaged cells to complete the process of degeneration. Remaining supporting cells participate in the formation of scars on the basilar membrane of the organ of Corti (Bohne and Clark, 1982).

Damage from noise exposure is cumulative, usually occurring gradually, and most of the time painlessly. Humans rarely know how much their ears are being injured until significant damage has been done. Most often the effects of acoustic over-exposure are not seen until late in life (Yost, 2000).

Environmental noise is a common cause of hearing loss in industrialized societies (Marcincuk and Roland, 2002), arising from exposures during both work and pleasure related activities (Maison and Liberman, 2000). According to the Occupational Health and Safety Administration, 5-10 million Americans are at risk for hearing loss because they are exposed to sounds greater than 85 dB on a sustained basis in the workplace

(Marcincuk and Roland, 2002). Ten million Americans have suffered irreversible noise-induced hearing loss due to exposure to dangerous noise levels each day (Noise and Hearing Loss, 1990). Moreover, approximately 48 million Americans engage in shooting sports, making this the most common cause of non-occupational noise-induced hearing loss (Marcincuk and Roland, 2002). Compared to people living in America, hearing in people living in unindustrialized societies—such as that of the Mabaans—is better from ages 10 through 70 (Bergman, 1966).

The amount of hearing loss occurring per person is related to the history of noise exposure experienced over the lifetime of the individual (Yost, 2000). Controversy exists regarding the percentage of presbycusis resulting from a lifetime of environmental noise-exposure and the percentage due solely from the physiological aging process (Marcincuk and Roland, 2002). Additionally, heritability estimates for genetic susceptibility to hearing loss following noise exposure are stronger than those for blood pressure or cholesterol levels (Gates et al., 1999). Known factors influencing noise-induced hearing loss are: intensity of the noise (dB), temporal pattern of the noise (continuous, intermittent, or transient), the frequency content of the noise, the duration of the exposure to noise, and individual susceptibility to noise (Marcincuk and Roland, 2002). Additionally, age at the time of excessive noise exposure influences hearing loss.

Sound levels of 75 dB are unlikely to cause permanent hearing loss in humans. However, levels over 85 dB with exposures of 8 hours a day will produce loss over time (Noise and Hearing Loss, 1990). The Sight and Hearing Research Association (2004) lists several examples of common noises that may, over time and with over-exposure, induce loss of hearing. These include a lawn mower (90 dB), snowmobile (100 dB), stereo

headphones (105-110 dB), rock concerts (115-120 dB), and firearms (125-140 dB). The recommended maximum exposure time for humans set by OSHA (Occupational Safety & Health Administration) is 8 hours per day at 90 dB (Marcincuk and Roland, 2002). For every 5 dB increase in noise volume, the maximum exposure time is cut in half. However, simply measuring the sound pressure level—or the loudness of a sound—does not assess the potentially damaging effects of noise because the human ear does not respond equally to all frequencies. High frequencies are much more damaging to the ear than low frequencies at the same physical intensity levels (Marcincuk and Roland, 2002). Over-exposure to noise can result in: acoustic trauma, *temporary threshold shifts* (TTS), *permanent threshold shifts* (PTS), and tinnitus (Takagi, Hiramatsu, & Yamamoto, 1988).

Damage to the ear can result from a single exposure or relatively few exposures to a very intense level of sound (Takagi et al., 1988). Noises causing this type of trauma are usually impulsive in nature, such as explosions. Acoustic trauma causes mechanical damage to the hair cells as well as to the supporting cells and tissues of the organ of Corti (Takagi et al., 1988).

Temporary threshold shifts are commonly experienced following exposure to sound levels over 70 to 75 dB (Takagi et al., 1988). Exposure to 115 dB or greater may pose a serious health risk (House Ear Institute: Advanced Hearing Science, 2005). These shifts in auditory thresholds return to normal between overexposures to intense noise because the hair cells recover from the stress. Sounds may seem temporarily muffled subsequent to these types of exposures. Depending on the nature of the exposure and the individual, temporary threshold shifts may last for minutes, hours, or even days post-exposure. In general, for continuous noise exposure, as the exposure time increases so

does the length and severity of the shift in threshold until after 4 to 12 hours of exposure, when a plateau is reached. The recovery subsequent to exposure is at first rapid, but then slows down, with complete restoration taking at least as long as the original exposure time. If intervals between these types of exposures are long enough for complete recovery, permanent damage is unlikely to occur. However, temporary threshold shifts are a warning sign that the auditory structures, particularly the hair cells and the stereocilia, are overloaded. Temporary threshold shifts are due to reversible biochemical changes occurring in the stereocilia of the hair cells (Takagi et al., 1988).

Permanent threshold shifts can occur gradually or with a rapid onset. In humans, it is high frequencies that are affected first, and because most of the speech frequencies are below this range the loss may initially go unnoticed. With further excessive noise exposure, however, the hearing loss extends to lower frequencies as well. Consequently, trouble understanding human speech may result (ISO, 1990).

There are many proposed mechanisms for permanent hearing damage to the inner ear structures. Stereocilia have been observed to lose their rigidity, due to destruction of their actin filaments (ISO, 1990). Damage to the rootlets anchoring stereocilia to the hair cell may also occur. Outer hair cells are more susceptible to damage than inner hair cells, because of the greater amount of displacement the stereocilia undergo due to their position at the base of the basilar membrane (ISO, 1990). With intense exposures of 140 dB or greater, a portion of the organ of Corti can be displaced from its position on the basilar membrane and float within the cochlear duct (Nordman, Bohne, & Harding, 1999).

Susceptibility to noise-induced hearing loss has a wide range of variability amongst humans. The same exposure to noise can result in responses varying from no hearing loss to large and debilitating losses in different individuals (ISO, 1990). Shifts in thresholds in response to noise may differ by as much as 30 to 50 dB among individuals (Noise and Hearing Loss, 1990).

This variability in susceptibility to NIHL occurs in mice as well as in humans. Studies have documented that there are both 'tough' and 'tender' ears, meaning that some individuals are more susceptible to damage than others. Analogous to this is the C57BL/6J mouse, which is more susceptible to noise-induced hearing loss than the CBA/CaJ strain (Davis et al., 1999).

Davis et al. (1999) found that inbred C57BL/6J mice were 5 to 10 dB more susceptible to noise than inbred CBA/CaJ mice. For the same level of noise, PTS was greater in the C57BL/6J mice than the control group. Groups of CBA/CaJ and C57BL/6J mice were exposed for 1 hour to different levels of noise ranging from 98 to 119 dB *noise spectrum level* (NSL) centered between 7 kHz and 17 kHz. The overall effects of noise exposure differences between C57BL/6J and CBA/CaJ mice were shown most clearly at 16 kHz, less at 32 kHz and a click stimulus, and least at 8 kHz.

Another study showed similar results (Jimenez et al., 2001). Female subjects were exposed at 3 months and 7 months to a 10 kHz octave band of noise at 105 dB SPL for one hour. Post-exposure thresholds were measured at 28 days. Results showed that CBA/CaJ thresholds were essentially unchanged, while C57BL/6J thresholds were significantly different when exposed at 3 months of age with an average loss of 20 dB. This difference was not seen in 7 month-old exposed C57BL/6J subjects. Thus, genetic

defects can predispose certain strains of mice to accelerated age-related hearing loss. Additionally, this study found that young C57BL/6J mice were more susceptible to noise exposure than older C57BL/6J mice.

The difference between strains in the susceptibility to noise-exposure is at least in part due to the *Ahl gene* (named for age-related hearing loss) that the C57BL/6J strain of mice possesses. Erway et al. (1996) examined a gene in mice thought responsible for loss of hearing, which was seen in humans with presbycusis. CBA/CaJ mice, which possess normal auditory function, were compared to C57BL/6J mice expressing the *Ahl* gene. Mice of both sexes and both strains were used in each testing group: a control group not exposed to noise, a group exposed for 1 hour at 110 dB, and a group exposed for 2 hours at 110 dB. The exposure consisted of a 1/3rd-octave band of noise centered at 5 kHz. There were significant differences in thresholds of hearing between CBA/CaJ and C57BL/6J mice at all frequencies tested post-exposure. The CBA/CaJ strain of mice exhibited only temporary threshold shifts, while the C57BL/6J mice showed permanent threshold shifts. Susceptibility to NIHL in C57BL/6J mice was due primarily to the *Ahl* gene, which is located on the tenth chromosome (Erway et al., 1996). Not only does this gene increase susceptibility to age-related hearing loss, but it is also responsible for increasing susceptibility to noise-induced hearing loss (Davis et al., 1999). Molecular biologists have discovered several other genes that predispose an individual to hearing loss (Yost, 2000; Willems, 2000).

Ohlemiller et al. (2000) asserted that the *Ahl* gene in C57BL/6J mice influences vulnerability to noise-exposure at 2 months of age. C57BL/6J mice were exposed to noise at either 1-2 months or 5-7 months. Noise exposure in this experiment consisted of a

broadband noise at 110 dB SPL for 23 to 240 minutes. Auditory brainstem responses from all animals were conducted 14 days post-exposure. Young animals (1-2 months) had higher exposure thresholds for noise-induced PTS than older animals (5-7 months). Thus, young mice—when compared to old mice—were more susceptible to noise-induced hearing damage, possibly due to the underdevelopment of structures in the auditory system.

When animals with noise-induced permanent threshold shifts were examined histologically, outer hair cell loss in the base of the cochlea was more widespread in the younger animals compared to older subjects. The C57BL/6J strain lost approximately 50% of OHCs when exposed at a young age. These data suggest that the mechanism or site of noise injury may differ between young adults and older adults (Ohlemiller et al., 2000).

In another study, two and eight-day-old guinea pigs of both genders were compared to 8-month-old guinea pigs exposed to 120 dB SPL of white noise for 30 consecutive hours. Hair cell damage in the organ of Corti was examined post-exposure. Results showed 23.72 percent damage in the 2-day old exposed subjects and 36.98 percent damage in the 8-day old subjects. Electrophysiological results from the 8-month old subjects showed hair-cell damage of only 7.24 percent (Falk et al., 1974). These data suggest that, in a species of animal other than mice, hearing loss is more severe when young mice are exposed to noise compared to older mice.

Dayal and Bhattacharyya (1986) compared 5 week-old female guinea pigs to 1-year-old guinea pigs following exposure to a 95 dB pure tone stimulus at 2 kHz. Subjects were exposed one hour a day for 5 weeks. This type of continuous exposure was

characterized as a chronic exposure of moderate intensity. Damage was assessed 3 weeks post-exposure by means of cochlear cell degeneration. Damage was worse for young animals compared to older animals. Coleman (1976) exposed 3 week, 10 week, and 49 week old guinea pigs to a 119 dB tone of 4 kHz for 2 hours. Younger guinea pigs had significantly more damage to the cochlea than the 8 month old subjects when assessed at 3 weeks post-exposure.

Li et al. (1993) showed contradicting results when female C57BL/6J and CBA/CaJ mice were exposed to a 120 dB SPL broadband noise for 5 minutes at 1, 2, 3, 6, and 12 months. Test of ABRs were conducted 1 month following exposure. Not surprising, C57BL/6J ABRs were more severely affected by acoustic trauma than were CBA/CaJ ABRs. While CBA/CaJ mice had an increased resistance to noise damage as a function of age, C57BL/6J mice showed a constant susceptibility with age. That is, C57BL/6J subjects were not less susceptible to noise exposure as a function of age (Li et al., 1993).

In a follow-up to the Shone et al. (1991) study, male C57BL/6J mice were exposed to a higher intensity of noise (108 dB SPL for 45 minutes). Mice aged 6 and 8 months were used, and thresholds were measured 1-2 weeks post-exposure in both groups. C57BL/6J mice showed increased sensitivity as a function of age to acoustic trauma due to their pathology of presbycusis (Shone et al., 1991).

Kujawa et al. (2003) were interested in the interactions between age-related and noise-induced hearing loss. CBA/CaJ mice were exposed to an 8-16 kHz *octave band noise* (OBN) at 100 dB SPL for two hours. (Octave band noise is a description used because most common sounds contain a wide range of frequencies, and it is easier to

divide this range into frequency bands using either full octave bands or the more detailed 1/3 octave bands. Each band is defined by its center frequency). Age grouped subjects were noise exposed at 4 weeks and 12-18 months. Of interest in the current study at NMU is not just the comparison of subjects, CBA/CaJ versus C57BL/6J, but the methodology of measuring thresholds at the same age post exposure rather than the same duration following exposure. Kujawa et al. (2003) questioned whether noise-induced hearing loss and age-related hearing loss was an additive or multiplicative effect. The young group (4 week old mice) of exposed subjects tested at 18-20 months of age experienced threshold shifts of almost 50 dB. In comparison, mice exposed at 4 weeks but tested 2 weeks post-exposure showed threshold shifts of only 25-30 dB. The relationship between AHL and NIHL is not merely additive, but is multiplicative or synergistic.

Miller et al. (1998) hypothesized that pre-existing damage resulting from presbycusis in addition to new damage following noise exposure would not add linearly. In their study, 8-month-old mice were more sensitive to noise exposure than 6-month-old mice. Six-month-old C57BL/6J mice showed an increase in threshold post-exposure of 30-40 dB at high frequencies, while 8-month-old mice showed a loss of thresholds of 35-45 dB at high frequencies. The author noted that it would be interesting to see what would happen to the hearing of 'young' versus 'old' experimental group thresholds if observed later (Miller et al., 1998). Thus, using the Kujawa et al. (2003) methodology of testing auditory thresholds at the same chronological age rather than same duration post-exposure may show different results (as detected by larger post-exposure threshold shifts between young and old mice when measuring at the same chronological age).

Most studies described above tested post-exposure thresholds almost directly following exposure to noise; e.g., days or weeks post-exposure. This means that subjects are tested at very different ages post-exposure. An alternative is to expose animals at young versus old ages, and then test all subjects at the same chronological age post-exposure. This would permit an evaluation of the interaction between the effects of time at noise exposure, and the aging process.

Hypotheses

The following hypotheses were tested in this experiment:

1) Corresponding to behavioral data obtained from our laboratory (Prosen et al., 2003), electrophysiological threshold shifts in C57BL/6J mice would increase as a function of age and would occur at high frequencies earlier than at low frequencies.
2) Hearing sensitivity as measured by changes in auditory thresholds would be the same in C57BL/6J mice regardless of their source: laboratory bred, vs. commercial vendor.
3) Auditory sensitivity as measured by changes in electrophysiologically measured ABR thresholds would be similar between male and female mice.
4) Subjects exposed at young ages would be more susceptible to acoustic over-stimulation than those exposed at older ages, when thresholds were assessed at the same chronological age.

METHODS

Subjects

Three groups of C57BL/6J subjects were used in this experiment. Two groups of C57BL/6J mice whose parents were obtained from the Jackson Laboratory were bred at the Northern Michigan University laboratory; one of these groups (N=10, 5 male and 5 female) was noise-exposed at 2 months of age, while the other group (N=10, 5 male and 5 female) was exposed at 6.5 months of age. The third group of C57BL/6J mice (N=10, 5 male and 5 female) was obtained from the Jackson Laboratory at 3-5 weeks of age; this group was not exposed to noise (See Appendix A). The third group of subjects in this experiment was used to assess the effects of long-distance travel-related noise damage on auditory thresholds. The 10 C57/BL/6J mice that were obtained from Jackson Laboratory, upon arrival, were quarantined for one week. Full access to food and water was maintained throughout the experiment for all subjects. All subjects were housed individually in 18 cm x 29 cm cages containing wood shavings. Cages were cleaned on a semi-weekly basis. The auditory brainstem response was measured in all subjects in the experiment at ages 2 and 7 months, while those mice exposed to noise at 6.5 months had ABR thresholds additionally recorded immediately prior to noise exposure. The Institutional Animal Care and Use Committee (IACUC) of Northern Michigan University approved the care and use of the subjects in this report (see Appendix C).

Apparatus

For the ABR procedures, silver chloride electrodes (A-M Systems, Inc.) were prepared as follows: 0.03 cm coated A-Mystiment Inc. electrodes were placed in an

opaque non-metallic container with a 5% salt (NaCl) solution. The electrode (connected to the positive terminal of a 1.5 – volt D battery) was immersed in a solution which created a difference in the electrode potential as evidenced by a darkened electrode tip.

ABR testing was performed in a controlled acoustical chamber manufactured by Industrial Acoustics Company. The foam-lined soundproof chamber had internal dimensions of 61 cm x 69 cm x 46 cm. Acoustic stimuli were calibrated by Microphone Power Supply (World Precision, Type 2804) and a microphone (Bruel & Kjaer, 2669). Activity from the electrodes led to an ISO-80 isolated Bio-amplifier (World Precision Instrument), which amplified the signal. A Dell Dimension Pentium 4 processor and software (written by Dr. Bradford May, Johns Hopkins University) was used during the ABR and noise-exposure procedures. A Crown D-75A Tucker Davis Technologies system was used for sound generation. A B&K Precision 200 MH2 Oscilloscope Model 2/20B was used. During the ABR signal, noise was filtered out with a Krohn-Hite filter (model 3500) in order to restrict the frequencies of background noise. Sound was calibrated with a Simpson 899 Type 2 impulse sound level meter. A halogen bright spot lamp (Burton) was used to monitor subjects. An Isotherm-heating (Deltaphase) pad was used to keep the subject warm.

Anesthetics

To anesthetize the subject for testing, subjects were given an intraperitoneal injection of a drug cocktail containing 0.5 ml xylazine, 1.0 ml ketamine, 0.625 ml ethanol, and 1.875 ml sterile H_20. Post-injection, the subject was observed until no whisker movement was detected and the subject had steady breathing. These physical

cues helped ensure that the subject was adequately anesthetized. Sterile ophthalmic eye ointment (Fougera, bacitracin - neomycin - polymycin) was administered by cotton tip applicator to keep the subject's eyes moistened during anesthesia. The subject was then placed on an isotherm-heating pad, which had been previously heated in a microwave oven. The tip of a lubricated probe thermometer was inserted rectally and taped in place across the tail with a piece of surgical tape. Core body temperature was maintained at approximately 37° C throughout the ABR procedure. If the temperature became too hot or too cold—defined as 37 degrees plus or minus 2 degrees—adjustments were made to shift the temperature accordingly.

Method of Measurement: Auditory Brainstem Response (ABR)

The auditory brainstem response (ABR) was used to measure hearing sensitivity in mice. This electrophysiological procedure, which provides a relatively noninvasive means of testing hearing, was a new measurement procedure in the Northern Michigan University Hearing Research Laboratory. Compared to behavioral testing, auditory thresholds can be obtained in a much shorter time period (approximately one hour).

The anesthetized subject was placed on its left side in the foam-lined IAC chamber. Silver chloride difference electrodes were then placed subcutaneously at two locations on the head, one dorsal and one ventral to the pinna. A third ground electrode was placed at the posterior midline of the skull. The subject's right ear was placed 5 cm directly below the speaker. A small pad was placed under the nose to ensure that the subject could breathe properly. The door to the chamber was closed, and the heart rate was monitored through external speakers throughout the entire ABR process.

Four types of auditory stimuli were presented—a click (including all frequencies), and 8, 16, and 32 kHz pure-tone signals. The order (8, 16, 32, and click) of presentation of stimulus type for each subject was randomized. The first stimulus was presented at 90 dB SPL. After presenting the stimulus, the next trial was *attenuated* by 10 dB, and the auditory response to this stimulus was measured. The stimulus was thereafter attenuated in increments of 10 dB until no auditory response was observed, evident by a visually assessed lack of peak to peak response (figure 5). Data were recorded and displayed on-line, with response magnitude shown at stimulus level (dB SPL) as a function of time. Thresholds were obtained prior to changing stimulus frequency. All auditory stimuli were 10 msec in duration, repeated at a rate of 30 pulses/sec, and responses were averaged over 1000 presentations. About a third of a second after initial presentation of the

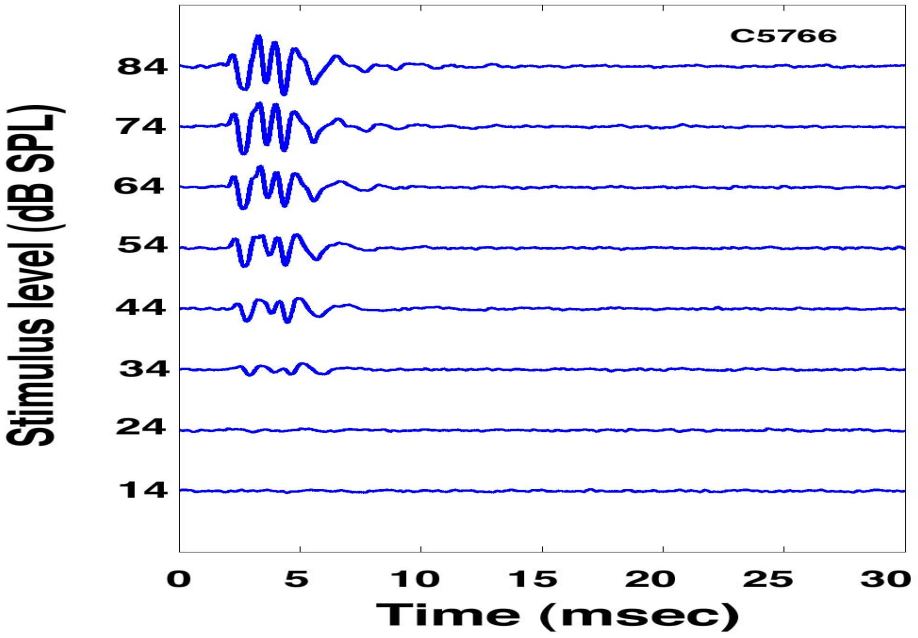

Figure 5. Shows ABR data of a female 2-month-old subject (C5766) to a click stimulus.

auditory stimulus, a response was evoked in the area of the brain where sounds are interpreted. The electrophysiological response, small and frequently masked by the "background noise" of electrical activity in the brain, was then averaged many times to eliminate background noise, leaving the neuro-psychological response of principal interest.

Threshold Shift Determination

Auditory brainstem response thresholds were defined as the tone level that produced a peak-to-peak voltage 2 standard deviations above the average background peak-to-peak levels, with the measurement determined in a 2.5 - 7.5-msec window post-stimulus onset and the background rate assessed 25 - 30 msec post-stimulus onset. As seen in figure 5, after attenuating a click tone stimulus by 10 dB per trial—from 80 dB to 70 dB to 60 dB, and so on—presentation of a 30 dB stimulus resulted in a minimal response. A miniscule peak-to-peak level in Figure 5 shows this. Notice from this figure that the response diminished at 34 dB and was only seen slightly at 24 dB. From this figure, a quick estimate can be established for the subject's threshold (between 34 and 24 dB SPL).Thus, using the defined calculation of threshold described above, the software calculated the actual threshold of 2-month old C5766 to a click stimulus was 21.4 dB SPL, as depicted in Figure 6.

Subject thresholds to all four stimuli were computed. In some instances, particularly 32 kHz post-exposure, thresholds could not be assessed because the hearing loss was too severe, particularly after exposure to noise, to measure any hearing. ABR thresholds were computed and analyzed for C57BL/6J male and female mice.

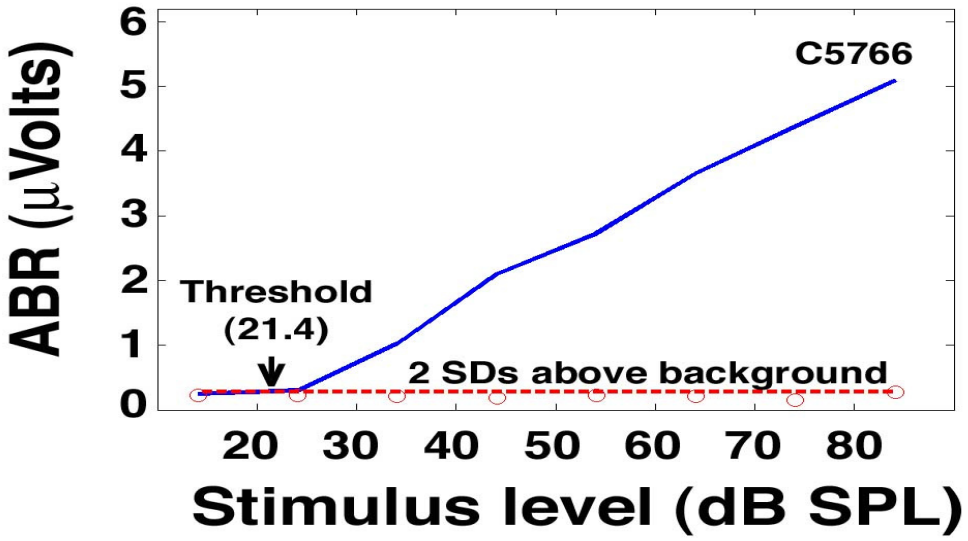

Figure 6. Shows calculation of ABR threshold to a click stimulus (subject C5766).

Once the ABR measurements were complete, the subject was then exposed to acoustic trauma. If the subject was not receiving noise-exposure and only thresholds were being measured, the subject was taken out of the testing chamber and the electrodes were removed. The subject was then returned to its home cage and monitored under a heat lamp until body movement was noted and was returned to the colony room.

Noise Exposure

Following ABR threshold determination, one group of NMU bred C57BL/6J subject was exposed at 2 months of age and the other group was exposed at 6.5 months. Noise exposures were conducted in the foam-lined IAC chamber. Subjects were still lightly anesthetized from the prior ABR procedure. When slight whisker movement was detected, the subject was ready for exposure. Whisker movement was an indication that the subject was awaking from anesthetic. An isothermal pad (Deltaphase) maintained body temperature at 37° C. The subject was placed in a ventral recumbency position

inside the exposure cage and put into the IAC chamber, with the subject's head located directly beneath the overhead speaker approximately 5 cm away.

The subject was binaurally exposed to a ½ octave bandwidth white noise centered at 11.2 kHz (101 dB NSL) for 76 min. To avoid the potential of inducing an audiogenic seizure, the noise increased in level over a 16 min interval, beginning at 80 dB. Noise for the exposure was calibrated by a Microphone Power Supply with a Bruel & Kjaer microphone. Immediately following the noise-exposure, the subject was taken out of the exposure chamber and placed in its home cage to recover, as described above for the post-ABR procedure, with full access to food and water.

Histological Analyses

To correlate the electrophysiological data obtained in the current study with changes at the hair cell level following noise exposure, subjects were transported to Johns Hopkins University, where they were euthanized and their cochleae perfused with fixation and retrieved from the temporal bones. Cochlear perfusions were completed under surgical anesthesia (125 mg/kg ketamine and 12.5 mg/ kg xylazine in 14.25 % ethanol). Histological evaluations were performed on sections of the organ of Corti. The cochleae were extracted from the temporal bones and placed in 0.1 % methyl - ethylene - diamine - tetra - acetic acid (MEDTA). Once decalcified, the cochleae were fixed in plastic to allow light microscopic analysis. Cochlear tissue was then evaluated via electron microscopy (Francis, Ryugo, Prosen, & May, 2003). These methods have been described in detail by Hequembourg and Liberman (2001). The results of histology are not part of this thesis.

RESULTS

1. Hearing loss as function of age

To assess hypothesis one, which suggested that C57BL/6J subjects would exhibit elevated hearing thresholds as a function of age and frequency, seven animals exposed at 6.5 months of age were used. These animals had two pretreatment hearing assessments, one at 2.5 months and one at 6.5 months of age just prior to noise exposure. A 2 x 4 (age x frequency) repeated measures Analysis of Variance (ANOVA) evaluated pre-noise thresholds. These data are displayed in Figure 7. A main effect of frequency was found, $F(3,18) = 11.114$, $p = .000$. There was also a main effect test age $F(1, 5) = 6.65$, $p = .042$. The interaction was not significant $F(3,18) = 2.49$, $p = .093$.

To examine the main effect of frequency, planned contrasts were used. The

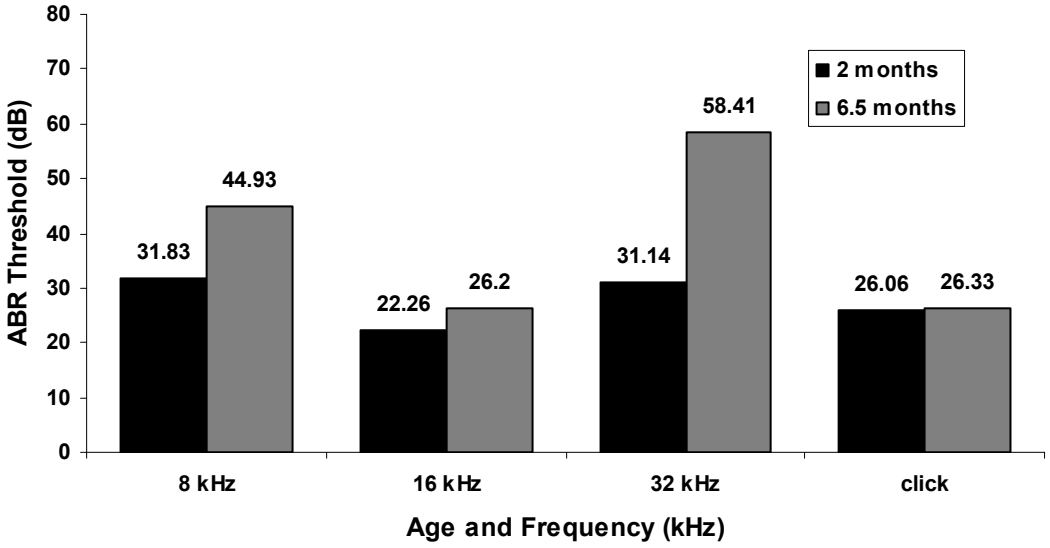

Figure 7. Shows comparison between average thresholds of 2 and 6.5 month-old C57BL/6J mice pre-noise exposure.

differences in frequency thresholds were evaluated using the click as a baseline because it included all frequencies. Overall, average threshold was lowest at 16 kHz (24.23 dB), not different from the click (26.19 dB), $F(1, 6) = .375$, $p > .05$. Thresholds at both 8 kHz (36.38 dB) and 32 kHz (44.93 dB) were significantly higher than thresholds at the click, $F(1,6) = 7.44$, $p = .034$, and $F(1,6) = 30.62$, $p = .001$ respectively. Pooled across frequencies, the younger animals had a lower threshold (*Mean (M)* = 27.82 dB) than the older animals ($M = 38.97$ dB).

While the overall age by frequency interaction was not significant, it was suggestive, $F(3,18) = 2.49$, $p = .093$, even with a small n. To further investigate the interaction, a Tukey's test was done and suggested that a change of 22 dB would be significant. Using this critical value, the overall change between 2 and 6.5 months was significant because all groups changed a little, but the only significant individual change was at the highest frequency of 32 kHz.

2. The Effects of Source on Hearing Loss

The question of whether transporting animals had an effect on hearing was addressed in a mixed model 2 (source) X 2 (sex) X 4 (frequency; repeated measure) ANOVA. Mice used were 10 JAX mice and 20 NMU mice pre-exposure. There was a significant effect of frequency using the Greenhouse-Geisser correction for lack of sphericity, $F(3, 41.86)$, $p = 0.006$. At two months, there was no effect of source, $F(1, 26) = 1.064$, of sex $F(1, 26) = 1.148$, and no significant interactions, all Fs = 1.84, $p > 0.15$.

To evaluate NMU versus JAX differences over time, a comparison of the animals that were tested pre-exposure at both 2 and 6.5 months of age was done using a 2 (source)

x 2 (sex) x 2 (age) x 3 (frequency) mixed model repeated measures ANOVA. This comparison included 8 JAX animals and 10 NMU animals. Only three frequencies (8 kHz, 16 kHz, and click) were used in this analysis because loss was too severe to measure any hearing thresholds at 32 kHz for several animals from both sources (figure 8). Thresholds at 32 kHz could not be measured in seven of eight Jackson animals and three of ten NMU animals. This seems to look like a differential dropout at 32 kHz between Jackson and NMU subjects, but a chi-square analysis of drop out was not significant, $X^2(1, N = 20) = 3.20, p > 0.05$. This analysis should be interpreted with caution due to the small *n*.

The ANOVA showed only an effect of frequency, $F(2, 28) = 31.71$, $p = 0.000$, test age, $F(1, 14) = 8.19$, $p = 0.013$, and an interaction of frequency and test age, $F(3,14)$

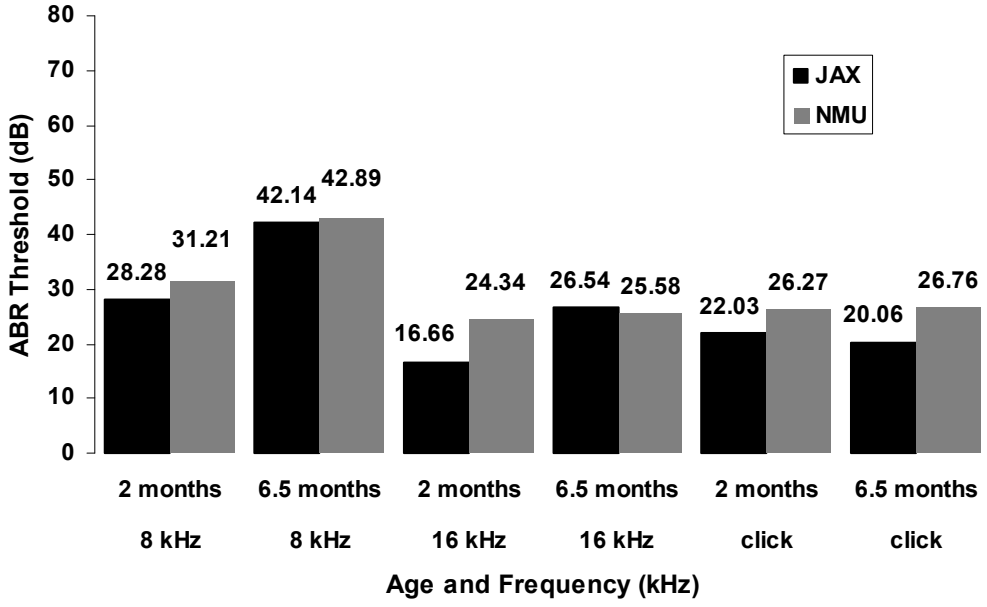

Figure 8. Shows comparison of NMU versus Jackson Laboratory bred C57BL/6J mice.

= 6.10, p = 0.01. There was no effect of source or sex and no other interactions, all Fs < 1.83, p > 0.198. The frequency effect shows a clear threshold elevation at 8 kHz and no change at 16 kHz or click. This comparison with 18 animals lends support to the argument stated above that there may be an age x frequency interaction that could not be detected with only seven animals.

To examine animals at 32 kHz, the NMU (n = 7) and JAX (n = 3) animals were compared in a 2 (source) X 2 (test age) mixed model ANOVA. There was only an effect of test age, $F(1, 8) = 9.72$, $p = 0.014$. There was not a significant effect of source and no interaction with age. With the small n, this must be interpreted with caution.

Figure 8 shows JAX and NMU thresholds as a function of subject source at 2 and 6.5 months, with threshold measured at all frequencies tested. While thresholds were elevated as a function of age, no significance differences between the two groups of subjects were noted under any stimulus condition. These data reject the hypothesis that effects of transportation noise interacted with age.

3. The Effects of Sex on Hearing Loss

To look at sex differences, separate analyses were done with animals at 2 and 6.5 months, because initial sex differences could be evaluated with a larger n at 2 months. Once the 2-month exposed animals were treated, they could not be included in the 6.5-month analysis. At 2 months there were 10 male and 10 female NMU animals and 5 male and 5 female JAX animals (N = 30). A 2 (source) x 2 (sex) x 4 (frequency) mixed model ANOVA found a significant effect of frequency, $F(3, 78) = 6.47$, p= 0.001, no effect of sex or source, and no interactions, all Fs < 1.90, p > 0.05 (figure 9).

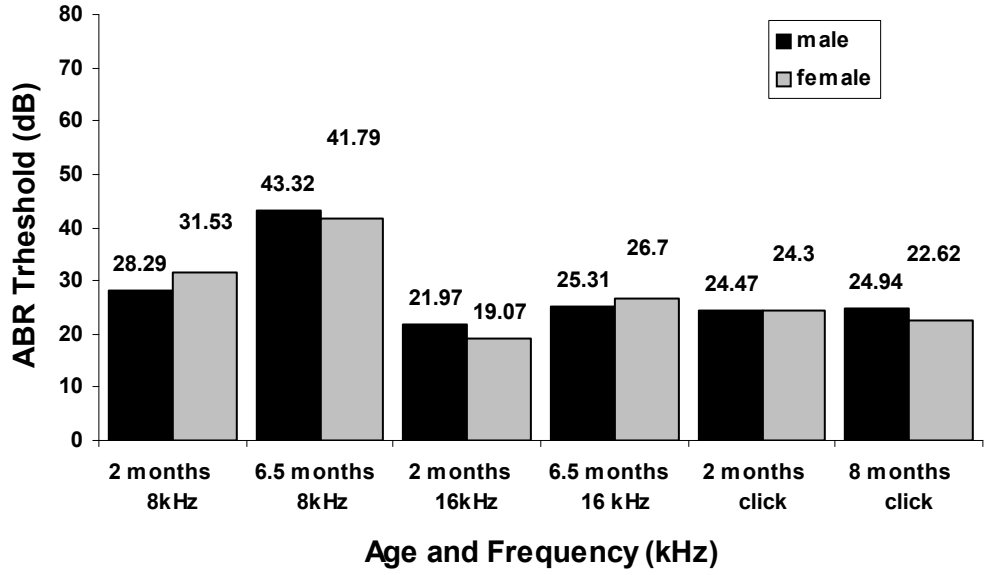

Figure 9. Shows sex comparisons of C57BL/6J mice at testing frequencies.

An apparent difference between males and females at 32 kHz was tested with independent *t* test and no difference was found $t\ (28) = 1.901$, $p > 0.05$, $p = 0.068$. The partial eta squared for sex x frequency in the analysis was 0.066 and power was 0.46. This suggests that if there was an initial effect at 32 kHz between males and females, it was small and small sample size does not provide enough power.

A change over time by sex at different frequencies was also of interest. Knowing there were no source differences, this variable was not included in the analysis. There were 9 male and 9 female with both 2 month and 6.5 month thresholds at 8 kHz, 16 kHz, and click. The drop in *n* from five at 2 months to four at 6.5 months in JAX animals was due to 2 animal deaths (1 male and 1 female) unrelated to experimental conditions. However, the 32 kHz threshold could not be established for 8 animals (3 NMU females, 1 NMU male, 1 JAX female, and 4 JAX male) at 6.5 months of age because their hearing loss was so severe that no threshold could be established.

Looking at 18 animals (9 female and 9 male) for whom thresholds could be established at 8 kHz, 16 kHz, and click in a 4 (frequency) x 2 (age) x 2 (sex) mixed model ANOVA showed there was a frequency effect $F(2, 28) = 31.648$, $p = 0.000$. There was also an age effect $F(1, 14) = 8.563$, $p = 0.010$. Additionally, there was a frequency by age interaction, $F(2, 28) = 6.290$, $p = 0.005$. The frequency effect reflects overall better hearing at 16 kHz and click and the age effect reflects poor hearing at 6.5 months compared to hearing at 2 months of age. There were no sex effects at 6.5 months of age. The frequency x age effect was discussed above. There were no significant threshold differences between females and males when tested pre-exposure at 2 or 6.5 months of age (Figure 9).

4. Loss of Hearing as a Function of Acoustic Overexposure

The question of whether the young or old ear is more susceptible to noise exposure was addressed using a mixed model 2 (age at exposure) x 2 (sex) x 3 (frequency) repeated measures Analysis of Variance. Mice used were 18 NMU C57BL/6J mice. Half of the mice were exposed at 2 months and half were exposed at 6.5 months of age. An equal number of males and females were used (9 females and 9 males). All thresholds used for this analysis were post-exposure thresholds taken at 7.5 months of age.

To compare post-exposure thresholds at the same chronological age rather than the same duration post-exposure, thresholds of mice exposed at 2 and 6.5 months (n=9 and n=9, respectively) were compared at the chronological age of 7.5 months. There was a significant effect of age at exposure $F(1,14) = 53.049$, $p = 0.000$. There were no gender

differences following exposure to noise $F(1,14) = 1.154$, $p = 0.301$, and no significant interactions $F(1,14) = 0.455$, $p = 0.511$.

Figure 10 shows 8, 16, and click data from 2 and 6.5 month exposed C57BL/6J subjects. Threshold shifts were: 37.13 dB, 32.61 dB, and 24.74 dB for stimuli of 8 kHz,

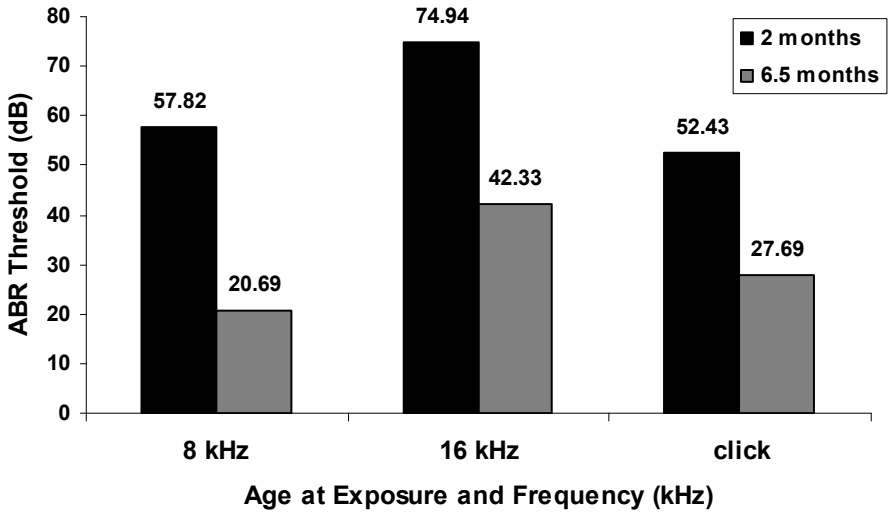

Figure 10. Shows comparison of thresholds of subjects noise-exposed at 2 months of age compared to those noise-exposed at 6.5 months of age.

16 kHz, and a click, respectively. No 32 kHz data were included because hearing loss at this age was too severe to measure via the ABR procedure. Thus, an apparent difference in hearing deficits at 32 kHz between 2 month and 6.5 month exposed C57BL/6J mice was assessed separately via a Chi square analysis in a 2 (age at exposure) x 2 (32 kHz) correlation of qualitative variables. The Chi square analysis showed that exposure to noise had an overall stronger effect on mice exposed at an early age ($X^2(1, N = 19) = 9.975$, $p > .002$). However, at 6.5 months, exposed mice had more cases of hearing loss at 32 kHz compared to mice exposed at 2 months of age. It seems that mice lose hearing

immediately at 32 kHz when exposed to noise at 6.5 months of age in comparison to exposure at 2 months of age.

Figure 10 shows the threshold changes or shifts for 8 and 16 kHz and click as a function of age at exposure. The figure shows that the same noise exposure was much more devastating to the young ear compared to the older ear. All frequencies tested when comparing young versus old age of exposure were significantly different.

DISCUSSION

1. Hearing loss as function of age

While C57BL/6J subjects demonstrate variability in hearing between subjects (Li and Borg, 1991; Prosen et al., 2003), the current study showed minor between-subject threshold variability. These data indicate in general that as subjects aged, hearing loss characteristic of presbycusis was apparent.

Most likely, a hearing loss at the lower frequency would have become apparent if subjects were tested after 6.5 months of age. Threshold shifts were least noticeable at 16 kHz, where mice are most sensitive to sound. A loss of hearing in C57BL/6J mice at low frequencies was described first by Hequenbourg and Liberman (2001), who suggested that low frequency threshold shifts reflects degeneration of fibrocytes in the apical portion of the cochlea, which extends into the scala vestibuli and scala tympani and forms a route of communication between the two structures.

The fact that thresholds at all frequencies were elevated at 6.5 months suggests the presence of histopathological processes throughout the cochlea. High frequency changes most likely reflect hair cell loss, with loss of spiral ganglion cells secondary to hair cell loss (Hequenbourg and Liberman, 2001).

Data from this part of the experiment suggested that the NMU Hearing Research Laboratory adequately measured and characterized the electrophysiologic changes characteristic of presbycusis in the C57 BL/6J mouse model. This was important to establish, since this was the NMU Hearing Research Laboratory's first experiment conducted using the ABR technique. The reliable results gained from comparing young

versus old C57BL/6J mice reinforced the use of this technique for the subsequent investigations described in this report.

2. Subject Source

One factor that might increase the variability of threshold changes amongst C57BL/6J mice is transportation noise incurred when young mice are moved from a commercial facility to the testing laboratory. The C57BL/6J inbred strain is the most widely used strain of mice in auditory research (Johnson et al., 1997). Thus, it is imperative to be certain that when laboratories receive these mice to use for auditory research, they are not arriving with a hearing loss from transportation sound exposure.

The C57BL/6J mice transported from The Jackson Laboratory had the same auditory sensitivity thresholds as did C57BL/6J mice born and raised in a sound-controlled laboratory setting (figure 8). The importance of these data is that researchers need not breed mice in their own laboratories to ensure normal hearing of their subjects prior to experimentation. This is a noteworthy finding given the current focus of testing transgenic animals, many of which are not widely available for use. Our findings were restricted to ground transportation; it would be of interest to conduct a similar study comparing hearing abilities of laboratory raised and air-transported young animal subjects.

3. Subject Sex

While the C57BL/6J mouse is recognized as an animal model of presbycusis, concern exists regarding which, if either, gender is a more appropriate model of the

human disorder. Human females are said to have a keener sense of hearing compared to human males (Barletta, 2003; Fisher, 1999; Howard, 2000; Stump, 1985). Alternatively, a recent report (Henry, 2002), suggested that age-related hearing loss proceeds faster in female C57BL/6J mice, but this effect was not found in this strain by Zheng et al. (1999).

In the current study, male and female subjects at 2 months of age showed differences in average thresholds of 3.92 dB at 8 kHz, 0.39 dB at 16 kHz, 4.66 dB at 32 kHz, and 1.18 dB to a click stimulus. These differences, especially with ABR measurements, are small and not significant. It has been said that females are less tolerant of loud noises and repetitive sounds (Stump, 1985). There was lack of inter-gender differences at test frequencies.

Both pre- and post-exposure auditory thresholds for males and females were measured in the current study. The fact that there were no differences in thresholds between sexes pre-exposure suggests that when exposure to noise is eliminated as a confounding variable, hearing of males is no different than that of females in the mouse model of presbycusis. While human females are said to hear better than human males, the discrepancy between the two is correlated with genetic and environmental factors, something this study controlled for.

4. Age at Noise Exposure

There is general agreement that the young murine ear is more vulnerable to noise than the older ear. Data from the current study concur with this observation. However, these data are unique because they evaluate hearing in an ear genetically predisposed to deafness when all subjects were evaluated at the same chronological age, versus the same

duration post-exposure. Early noise-induced cochlear damage seems to predispose the ear to accelerated degenerative changes later in life. Subjects may experience an early acoustic insult and then improve or recover from the damage, only to show hearing impairments later in life. Another possibility is that the individual may not have completely recovered from the exposure.

In humans, early exposure may not leave the person aware of a hearing loss, but presbycusis may become apparent with age-related cochlear degeneration at middle age. Contrary to Dobie (1985), the current study demonstrated an interaction between noise-induced loss and presbycusis that was not just additive. A simple additive model cannot explain the larger shifts in threshold observed when subjects' experienced acoustic trauma at a young compared to an older age. Interestingly, when exposed at an early versus mature age, mice were affected most at the frequency of greatest sensitivity—16 kHz.

It has been hypothesized that the site of damage resulting from noise may differ in young versus mature animals (Ohlemiller et al., 2000). The site of this difference can be studied by examining noise-induced hair cell loss in the cochlea.

The human cochlea and peripheral sensory end organs complete their normal development by 24 weeks of gestation, but are not fully mature at this time. Thus, exposure of the fetus and newborn to noise occurs during the normal development and maturation of the auditory system. Since sound is well transmitted into the uterine environment, this can cause damage to underdeveloped acoustic structures (Pediatrics, 1997).

Experimental evidence from the current study suggested that acoustic trauma incurred early in life is associated with greater sensorineural hearing loss as age increases. Many studies have documented hearing loss in children cared for in the Neo-natal Intensive Care Unit because they are exposed to continuous loud noise, particularly from an incubator (Kam, Kam, & Thompson, 1994; Lary, Briassoulis, de Vries, Dubowitz, & Dubowitz, 1985; Stennert, Schulte, Vollrath, Brunner, & Frauenrath, 1978; Winkel, Bonding, Larsen, & Roosen, 1978). Moderate-to-severe sensorineural hearing losses were also seen in infants treated with the ototoxic drug kanamycin. These infants were kept in incubators, suggesting an interactive response with exposure to noise and the ototoxic drug (Winkel et al., 1978).

Approximately half of 56 incubator-treated children had minor changes in their audiograms suggesting minor noise-induced cochlear lesions (Stennert et al., 1978). This loss could be accentuated if the individual carried an age-related-hearing loss gene. The US Environmental Protection Agency has suggested that a noise level for young infants exceeding 45 dB is best avoided (Committee on Environmental Health, 1997).

CONCLUSIONS

Data from these experiments support the following conclusions:

1) Corresponding to behavioral data obtained from our laboratory, electrophysicologic threshold shifts in C57BL/6J mice increased as a function of age and occurred at high frequencies (32 kHz) prior to changes at lower frequencies.
2) Subject source (laboratory vs. commercial vendor) influenced neither initial thresholds nor those measured at middle age in an ear genetically predisposed to hearing loss. Hence, ground transportation noise in a "tender" C57BL/6J ear is not an important consideration in studying this animal model of presbycusis.
3) Male subjects had comparable hearing to female subjects. Thus, both genders are acceptable models of the human hearing disorder.
4) Young subjects were particularly sensitive to the effect of acoustic over-stimulation compared to older subjects. This difference may have been pronounced in this experiment compared to data from other studies because threshold shifts in mice were measured at the same chronological age rather than the same duration post-exposure.

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APPENDIX A

TABLE OF ALL DATA USED FOR ANALYSES

ABR#	Sex	Source	Exposure Age	2m 8kHz	2m 16kHz	2m 32kHz	2m Click	6.5m 8kHz	6.5m 16kHz	6.5m 32kHz	6.5m Click	7.5m 8kHz	7.5m 16kHz	7.5 m 32k hz	7.5 m click
60	M	NMU	6.5 mo	41.4	31.9	22	25	81.3	36.8	68	25.6	59.6	60.1	60.4	48.5
61	M	NMU	6.5 mo	25.8	25.1	30.8	26.8	33.1	-4.8	53.3	20.3		70.6		39.4
62	M	NMU	6.5 mo	49.5	33	36.6	30.9	42.8	14.6	63.4	29.3	42.8	69.4		45.4
70	M	NMU	6.5 mo	27.6	18.1	35.3	24.3	18.7	30.9	72.9	22.5	41.3	59.7	52.5	57.3
71	M	NMU	6.5 mo	33.7	13.6	56.1	29.9	49.7	56.5	31.3	25.8	58.1	70.9		57.4
63	F	NMU	6.5 mo	21.9	20.8	27.2	26	34.3	27.0		22.7	59.0	67.5		64.9
64	F	NMU	6.5 mo	31.5	31.4	2.9	27.3	46.2	23.9		33.7	56.5	66.4		48.4
65	F	NMU	6.5 mo	22.2	4.4	16.8	24.1	45.5	30.0	80.1	30.6	52.1	77.5	49.5	65.5
66	F	NMU	6.5 mo	22.6	29.7	20.4	21.4	43.4	19.4	39.9	30.2	38.2	52.8		37.2
67	F	NMU	6.5 mo	35.9	28	23.8	27	33.9	21.5		26.9	64.9	67.6		60.5
72	F	NMU	2 mo	24.6	1.4	23.3	26.8					81.0	86.0	74.0	80.3
73	M	NMU	2 mo	37.4	19.8	30.4	25.6					75.7	86.0	74.0	88.0
74	M	NMU	2 mo	24.7		31.1	14					85.4	73.2	74.0	73.0
75	M	NMU	2 mo	13.6	10.6	30.7	22.7					91.0	86.0	74.0	85.2
76	M	NMU	2 mo	25.5	17.6	21.6	26.4					79.5	86.0	74.0	72.2
78	M	NMU	2 mo	19.9	6.3	22.8	18.8					78.3	92.0	76.0	65.2

79	**F**	**NMU**	**2 mo**	**19.3**	**5.3**	**12.7**	**20.3**					**85.1**	**92.0**	**76.0**	**69.9**
81	**F**	**NMU**	**2 mo**	**33.6**	**28.4**	**5.3**	**22.8**					**88.8**	**92.0**	**76.0**	**54.4**
82	**F**	**NMU**	**2 mo**	**25.7**	**1.8**	**32.3**	**19.0**					**79.9**	**92.0**	**86.0**	**80.0**
83	**F**	**NMU**	**2 mo**	**24.7**	**14.1**	**56.2**	**15.6**								
84	**F**	**JAX**		**23.9**	**30.3**	**28.1**	**25.6**	**36.8**	**30.6**	**56.1**	**12.8**	**33.0**	**44.1**	**86.4**	**27.0**
88	**F**	**JAX**		**33.9**	**24**	**14.1**	**27.0**	**58.1**	**32.6**	**58.7**	**28.6**	**41.4**	**46.4**		**34.7**
89	**F**	**JAX**		**33**	**14.9**	**1.5**	**20.8**	**50.6**	**12.5**	**11.1**	**15.2**	**32.0**	**22.4**	**31.0**	**23.3**
91	**M**	**JAX**		**29**	**13.8**	**22.6**	**19.9**	**31.6**	**23.6**		**14.3**	**35.4**	**12.4**	**74.0**	**25.2**
94	**M**	**JAX**		**29**	**10.7**	**53.2**	**18.6**	**35.8**	**27.5**		**16.5**	**40.6**	**30.4**		**31.7**
95	**M**	**JAX**		**18.4**	**11.4**	**26.5**	**16.9**	**35.0**	**22.5**		**17.3**	**30.6**	**30.8**		**23.2**
96	**M**	**JAX**		**29.4**	**14**	**16.1**	**26.4**	**48.1**	**32.7**		**32**				
97D	**M**	**JAX**		**34.5**	**21.3**	**4.2**	**24.9**								
98	**F**	**JAX**		**29.7**	**14.2**	**16.4**	**21.0**	**41.1**	**30.3**		**23.8**				
103D	**F**	**JAX**		**29**	**17.2**	**20.3**	**22.3**								

Note. The letter D following ABR# annotates animal death. Blank spaces within cells mean no data were collected for that stimulus.

APPENDIX B

PERMISSION TO USE FIGURES VIA ELECTRONIC CORRESPONDENCE

Director of Research
School of Communication
Simon Fraser University
Burnaby, B.C.
April 5, 2007

Barry Truax,

Hello! I am currently a graduate student at Northern Michigan University and am finishing my thesis titled: Effects of source, gender, and age at sound exposure on hearing in C57BL/6J mice.

I am interested in using a figure of a diagram of the cochlea that I found on the website: http://www.sfu.ca/sonic-studio /handbook/Cochlea.html, and located your name from that site as well. Would I be able to obtain permission from you to include this figure in my thesis?

I am set to defend my thesis on April 27, 2007. I would appreciate a response about this, and am extremely excited to be finishing up with the master's program here in Michigan. Thank you in advance, and thank you as well for your interest and effort in studying the auditory system. I look forward to hearing from you!

Sincerely,

Alyssia Rogers

Date: Thu, 5 Apr 2007 23:39:40 -0700
From: "Barry Truax" <truax@sfu.ca>
To: "Alyssia Rogers" <alrogers@nmu.edu>
Subject: **Re: Graduate Student**

Yes, you may use the Cochlea diagram from the Handbook. Note that the byline includes the reference to the original source, Stevens' Sound and Hearing, which you should probably include as well. But since the actual diagram is one which we created, I think my permission is all that is needed. Please refer to the source as the Handbook for Acoustic Ecology, B. Truax, ed., CD-ROM, Cambridge Street Publishing, 1999.

All the best

Barry

website: www.sfu.ca/~truax

Alyssia Rogers
1216 Cooper Lake Rd.
Ishpeming, MI 49849
April 5, 2007

Dr. David Furness,

Hello! I am currently a graduate student at Northern Michigan University and am finishing my thesis titled: Effects of source, gender, and age at sound exposure on hearing in C57BL/6J mice.

I am interested in using a figure an electron micrograph of a sensory hair cell and hair bundle that located on the website: www.keele.ac.uk/depts/co/auditory/ages/projects.htm and found your name from that site as well. Would I be able to obtain permission from you to include this figure in my thesis?

I am set to defend my thesis on April 27, 2007. I would appreciate a response about this issue, and am extremely excited to be finishing up with the master's program here in Michigan. Thank you in advance and thank you as well for your interest and effort in studying the auditory system. I look forward to hearing from you!

Sincerely,

Alyssia Rogers

Date: Fri, 6 Apr 2007 09:02:25 +0100 (BST)
Subject: **Re: Graduate Student**
From: d.n.furness@cns.keele.ac.uk
To: "Alyssia Rogers" <alrogers@nmu.edu>

Dear Alyssia

I am happy to give you my permission to use these images. Please note though that they are vestibular rather than auditory hair cells. I can send you similar images of auditory ones if you prefer.

Good luck with your thesis!

Best wishes
Dave Furness

Date: Sun, 6 May 2007 22:20:07 -0400 (EDT)
From: "Alyssia J Rogers" <alrogers@nmu.edu> **Block Address**
To: thib@utdallas.edu
Subject: **Alyssia (NMU graduate student)**

Dr. Linda Thibodeau

Hello! I am currently a graduate student at Northern Michigan University and am finishing my thesis titled: "EFFECTS OF SOURCE, GENDER, AND AGE AT SOUND EXPOSURE ON HEARING IN C57BL/6J MICE".

I am interested in using a figure of a diagram of the outer, middle, and inner ear that I found on the website: http://www.utdallas.edu/~thib/rehabinfo/te.htm, and located your name from that site as well. Would I be able to obtain permission from you to include this figure in my thesis?

I defended my thesis on April 27, 2007, and am extremely excited to be finishing up with the master's program here in Michigan. Thank you in advance, and thank you as well for your interest and effort in studying the auditory system. I look forward to hearing from you!

Sincerely,

Alyssia Rogers

Subject: **RE: Alyssia (NMU graduate student)**
Date: Tue, 8 May 2007 00:02:49 -0500
From: "Thibodeau, Linda K" <thib@utdallas.edu> **Block Address**
To: "Alyssia J Rogers" <alrogers@nmu.edu>

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214-905-3108
Fax: 214-905-3146

Yes, you have permission to use the figure. Good luck with your project!

LT

Subject: **RE: Thesis Project**
Date: Thu, 5 Apr 2007 14:52:32 -0500
From: "Oghalai, John Steven" <jso@bcm.tmc.edu>
To: "Alyssia Rogers" <alrogers@nmu.edu>

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jso@bcm.edu
http://www.bcm.edu/oto/jsolab

You are welcome to use them, as long as the work is acknowledged.

Thanks,

John S. Oghalai, MD

APPENDIX C

APPROVAL TO USE VERTEBRATE ANIMALS

Northern Michigan University

Continuing Education & Sponsored Programs
1401 Presque Isle Avenue
Marquette, MI 49855-5325

January 15, 2004

TO: Cindy Prosen
Psychology Department

FROM: Leonard Heldreth, Associate Provost
Academic Affairs

RE: **Application to use Vertebrate Animals**
Application # IACUC 005 (Modification)
Approval Period: April 1, 2004 – March 31, 2005

The Institutional Animal Care and Use Committee has approved your application to use vertebrate animals in research entitled "Auditory Processing of Signals in Noise."

If you have any questions, please contact me.

ljh

cc: Psychology Department

Telephone: 906 227-2102 ■ FAX: 906 227-2108