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PROTECTIVE EFFECTS OF AUDITORY TOUGHENING ON NOISE INDUCED HEARING LOSS IN RATS

By

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THESIS APPROVAL PAGE

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ABSTRACT

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Cynthia A. DeMots

Exposure to a sound provides protection against a subsequent traumatic sound. This toughening effect was studied in rats using different levels of stimuli. Rats were exposed to a 60 dBA or 72 dBA broad band noise for 5 days, 12 hours per day. Recovery from a 110 dB SPL 7.8 kHz pure tone presented for 20 minutes was measured using auditory brainstem response thresholds and distortion product otoacoustic emissions immediately after the traumatic exposure and 10 to 12 days later.

Auditory toughening did not have an effect on hearing and outer hair cell function immediately following a traumatic sound exposure. The toughened groups showed, however, a greater recovery than the control group, which was proportionally dependent on the toughening sound intensity, 10 to 12 days after the traumatic sound exposure.

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CHAPTER 1

INTRODUCTION

Damaging effects of noise exposure, which may cause hearing loss have become more recognized in society. While this type of hearing loss is almost completely preventable, it is still extremely prevalent. Extensive noise exposure damages the outer hair cells (OHCs) and the inner hair cells (IHCs) in the cochlea, resulting in a noise induced hearing loss (NIHL). The intensity, frequency, and duration of the exposure determine the degree of hair cell damage. Noise induced hearing loss is sensorineural in nature and typically affects high frequency hearing (3 kHz – 6 kHz). This type of hearing loss is characterized by a notch centered at 4 kHz with better hearing sensitivity at lower and higher frequencies (Bilger, 1976).

Hearing protection devices such as ear plugs and ear muffs are recommended to protect individuals from damaging noise in and out of the workplace. Other strategies that are being researched include the use of oto-protective agents such as magnesium (Scheibe, Haupt, & Ising, 2000), which could be combined with anti free radical substances (La Prell, 2007) as well as exposure to non-damaging sounds in order to protect the cochlea from changes that occur during exposure to damaging noise (Canlon, 2002). Recent studies have found that pre-conditioning techniques successfully protect the inner ear from damaging levels of sound (Canlon, Borg, & Flock, 1988; Canlon & Fransson, 1998).

Animals previously exposed to a low level non-damaging noise exhibit a smaller threshold shift following a subsequent exposure compared to animals not previously exposed to a low level noise. There are two main hearing protection paradigms that may assist the cochlea in being resistant to the effects of acoustic trauma: sound conditioning and auditory toughening. Sound conditioning uses a continuous stimulus, whereas auditory toughening involves an interrupted stimulus. Various parameters of the noise have been used for each test paradigm, which are discussed in detail in Chapter 2. However, multiple variables such as: a) constant noise versus interrupted noise, b) duration of the sound conditioning stimulus, c) frequency of the sound conditioning stimulus, d) intensity of traumatic exposure, and e) period of rest between sound conditioning and traumatic noise, make it difficult to determine which test paradigm and sound parameters yield the most protection from damaging noise (Niu & Canlon, 2002).

Research is limited with regard to the amount of previous exposure needed and the intensity at which the sound conditioning should occur. Since these studies cannot ethically be conducted on humans, an animal model has been used to study the damaging effects of noise. The purpose of this study is to further explore the effect of auditory toughening on the cochlea and hearing in rats, more specifically the lowest intensity in which protection is still afforded from a subsequent traumatic exposure.

CHAPTER 2

REVIEW OF LITERATURE

Noise Induced Hearing Loss

Hearing loss impacts millions of people worldwide. Approximately 28 million people in the United States have some degree of hearing loss as a result of hair cell loss. More than 30 million Americans experience exposure to damaging sound regularly (National Institute on Deafness & Other Communication Disorders [NIDCD], 2002). It has been estimated that the annual cost of untreated hearing loss in the United States is more than $100 billion over a one year period (Hearing loss, 2005).

There are several identified causes of hearing loss. These include, but are not limited to, heredity, age, ototoxic medications, and noise exposure. Genetic forms of hearing loss can be either congenital or progressive. There are many genes that have been found to cause hearing loss or deafness. Presbycusis, hearing loss related to aging, results from a variety of factors; however, the main contributing factor is heredity (Phillips, 2003). There are a variety of ototoxic medications that cause temporary or permanent hearing loss. These medications include antibiotics, diuretics, analgesics, and antineoplastic drugs (Konrad-Martin, 2005). Exposure to noise is a frequent cause of hearing loss. Noise exposure can lead to damage of the outer and inner hair cells in the cochlea (Gelfand, 2001). Hearing loss caused by ototoxic medications and noise exposure can likely be prevented (Roizen, 2003).

Noise induced hearing loss is the second most common type of sensorineural hearing loss (SNHL), accounting for approximately one-third of all hearing loss cases. The most common type of SNHL is presbycusis (NIDCD, 2002).

Susceptibility to NIHL varies among individuals. Some factors affecting susceptibility include the effectiveness of the acoustic reflex and previous history of noise exposure. Non-auditory factors include ototoxic drugs, environmental toxins, age, gender, eye color, and smoking (Gelfand, 2001). Noise induced hearing loss is characterized by a SNHL in the 4 kHz frequency range with better hearing at frequencies above and below 4 kHz. This is referred to as a "noise notch" (Bilger, 1976).

Noise trauma is harmful to the auditory system, more specifically, the inner ear. Exposure to loud sounds over time can produce threshold shifts in hearing sensitivity as a result of overstimulation to the cochlea (Gelfand, 2001). These threshold shifts can be temporary or permanent and are sensorineural in nature. A short-term change in hearing sensitivity induced by noise is known as a temporary threshold shift (TTS). Noise intensities greater than 80 dB SPL can produce a TTS. The intensity and duration of the stimulus directly affects the degree of TTS. As the intensity and duration of the stimulus increases, the TTS increases. This results in a longer recovery period. A permanent threshold shift (PTS) occurs when there is a change in hearing sensitivity caused by exposure to noise that does not return to previous hearing thresholds. Exposure to loud sounds has been known to produce a PTS, resulting in some degree of NIHL. There are many factors that determine the degree of PTS. These include the frequency, intensity and duration of the acoustic signal; the duration of the exposures over time; and the individual's susceptibility to noise exposure (Bilger, 1976).

Exposure to hazardous noise can cause a temporary or permanent threshold shift. Noise induced hearing loss can be caused by one exposure to damaging sound or multiple exposures over time (Gelfand, 2001). Prevention of NIHL includes reducing exposures to hazardous noise and wearing hearing protection devices (Berger & Lindgran, 1992).

Noise exposures can be characterized as recreational noise or occupational noise. Recreational activities that may cause a NIHL include firearms, woodworking, motorcycles, and other noisy pastimes. Individuals are often exposed to loud sounds in the workplace. The Occupational Safety and Health Administration (OSHA), a subdivision of the United States Department of Labor, has regulations regarding protection against noise exposure in the workplace. The Occupational Safety and Health Administration has enforced these regulations in order to reduce the degree of hearing loss caused by occupational noise. According to the Occupational Noise Exposure standard 1910.95, when employees are exposed to noise levels that exceed a certain level for a given period of time it is mandatory that employers provide hearing protection devices for these individuals (Occupational Safety and Health Administration, 1983). Table 1 outlines the amount of time in hours per day that an individual can safely be in an environment with a certain intensity level (in dBA) without expecting a threshold shift.

Table 1. OSHA Regulations for Noise Exposure (OSHA, 1983)

Hours per day	Sound level (dBA)
8	90
4	95
2	100
1	105
.5	110
.25 or less	115

If the individual is exposed to a specific intensity that exceeds the amount of time listed in Table 1 it is suggested that the work environment be changed to lower the intensity or duration of the exposure. If the sound level cannot be reduced to the appropriate levels listed in Table 1, hearing protection devices must be provided. All work environments in which employees are exposed to noise exceeding 85 dBA in an eight hour period are required to implement a hearing conservation program. This program monitors the employees' hearing thresholds annually. In addition, an educational program must be implemented to inform employees of the potential danger to their ears (OSHA, 1983).

Hearing Protection

There are a variety of ways to protect the ear from the damaging effects of noise. Currently, these include hearing protection devices and educational programs. Hearing protection devices include earmuffs and earplugs. These devices prevent individuals from being exposed to dangerous levels of noise; however, they also make it more

difficult to communicate with others while wearing the hearing protections devices. For this reason employees are resistant to wearing hearing protection. In some cases, hearing protections devices may introduce a problem of overprotection in the workplace. The use of hearing protection devices may cause employees to misunderstand or not be able to hear important instructions (Berger & Lindgren, 1992).

It is also important to inform individuals about the dangers of being exposed to damaging sounds. Hearing conservation programs provide education about NIHL and the use of hearing protection devices. The implementation of hearing conservation programs help prevent NIHL (Zohar, Cohen, & Azar, 1980).

Another potential way to protect the ear from damaging sound includes previous exposure to sound. An individual's susceptibility to NIHL may be affected by previous history of noise exposure. Prior exposure to a low level sound has been shown to reduce the threshold shift following a traumatic sound in animals. In chinchillas, a reduction in threshold shift can be as much as 20 to 30 dB when compared to a control group that was only exposed to the traumatic sound (Campo, Subramaniam, & Henderson, 1991). This study included 14 chinchillas, 6 animals in the experimental group and 8 animals in the control group. The experimental group was exposed to octave band noise (OBN) centered at 500 Hz at an intensity of 95 dB SPL for a period of 6 hours per day for 10 days. Five days after conditioning, both groups were exposed to OBN centered at 500 Hz at 106 dB SPL for 48 hours. Initially, the experimental group exhibited approximately 20 to 30 dB less of a threshold shift between 500 Hz and 4 kHz when compared to the control group. Five days after the traumatic exposure recovery was complete. There was a significant difference in the PTS between the experimental and the control groups.

There are a variety of situations in which hearing protection devices do not provide enough protection or hearing protection devices are not feasible. Hearing protection devices may not provide enough protection to individuals in the military, fire department, and police force. In these professions there are also instances in which hearing protection devices are not available at the time of exposure to a damaging noise. It has been hypothesized that another hearing protective strategy may be beneficial to these individuals and may help protect their ears from damaging noise. Sound conditioning/auditory toughening is a potential form of hearing protection, which allows an individual to be exposed to increased levels of noise without experiencing significant damage to the hearing system (Niu & Canlon, 2002; Prasher, 1998). In order to better understand the effects of sound conditioning/auditory toughening on the cochlea, the anatomy and physiology of the ear will first be discussed.

Anatomy and Physiology of the Normal Peripheral Auditory System

The auditory system is divided into two parts: the peripheral auditory system and the central auditory system. The peripheral auditory system is comprised of the outer, middle, and inner ear. The central auditory system consists of the VIII cranial nerve, auditory pathways in the brainstem, and the auditory cortex. The main focus of this section will be on the inner ear, as that is typically where effects of noise damage occur.

Outer and middle ear anatomy and physiology. The ear consists of three parts: the outer ear, the middle ear, and the inner ear. The outer ear is comprised of the pinna and the external auditory meatus, also known as the ear canal. The pinna funnels sound into the ear canal toward the tympanic membrane and aids in sound localization (Hebrank & Wright, 1974; Shaw, 1974).

The middle ear consists of the tympanic membrane, tympanum, and three bones known as the ossicles. These three bones are the malleus, the incus, and the stapes (Tonndorf & Khanna, 1970). Vibrations of the ossicles cause the stapes to move in and out of the oval window, a membrane of the inner ear. This movement causes the fluids in the inner ear to move. The impedance of the air filled middle ear is less than the impedance of the fluid filled cochlea. The middle ear allows transmission of the sound energy to the cochlea without loss of energy acting as an acoustic impedance transformer (Bekesy, 1960).

Inner ear anatomy. The inner ear is composed of the cochlea, vestibule, and semicircular canals. The cochlea is related to the auditory system, whereas the vestibule and semicircular canals are related to the vestibular system (Schubert, 1980).

The cochlea is a spiral shaped bony structure that is thickest at the base and thinnest at the apex (Yost, 2000). The cochlea consists of two and three quarter turns around the modiolus, or bony core. Within the cochlea there are three fluid filled areas: the scala vestibuli, the scala media, and the scala tympani. The scala vestibuli and scala tympani contain perilymphatic fluid, whereas the scala media is filled with endolymphatic fluid. The scala media separates the scala tympani and the scala vestibuli. The scala vestibuli is separated from the scala media by Reissner's membrane and the scala tympani is separated from the scala media by the basilar membrane which is narrow at the base and gradually widens toward the apex (Rappaport & Provencal, 2002).

The organ of Corti, also known as the sense organ of hearing, is located on top of the basilar membrane and below the tectorial membrane in the scala media. This structure contains sensory hair cells and a variety of supporting cells. Sensory hair cells

include inner hair cells (IHCs) and outer hair cells (OHCs). Each sensory cell contains tiny hairs located on top called stereocilia (Davis, 1962). The stereocilia of OHCs are attached to the tectorial membrane, whereas the stereocilia of IHCs are loosely coupled to the tectorial membrane (Lim, 1986).

There is one row of IHCs consisting of approximately 3,500 hair cells and three rows of OHCs containing approximately 12,000 hair cells (Davis, 1962). The shape and function of IHCs and OHCs are vastly different. The OHCs are cylinder shaped and the IHCs resemble a flask. The intracellular components of OHCs are located along the top and bottom of the cell, whereas the intracellular components of IHCs are distributed evenly throughout the cell. Both OHCs and IHCs have bundles of stereocilia, tiny projections, located on top of each hair cell (Davis, 1962). Each OHC contains approximately 150 stereocilia which are separated into bundles of three to four rows of different heights. The tallest row of stereocilia is rooted in the tectorial membrane and the other rows are connected to each other via cross-links (Lim, 1986). There are three types of crosslinks; tip-to-tip crosslinks, side-to-side crosslinks, and tip-to-side crosslinks (Gelfand, 1998). These cross-links are crucial to the transduction process. Unlike OHCs, each IHC only contains 50 to 70 stereocilia which are thicker than OHC stereocilia (Davis, 1962).

The auditory signal is transmitted from the hair cells to the brainstem by way of the VIII cranial nerve, also known as the auditory nerve. The auditory nerve contains afferent and efferent neurons. The afferent neurons send information from the hair cell to the auditory cortex, whereas the efferent neurons send information from the superior olivary complex down to the hair cells. Ninety five percent of auditory nerve fibers

contact IHCs (Gelfand, 2001). There are two efferent bundles of neurons: medial and lateral. Medial efferent neurons, which are the majority of efferent neurons, come from the medial nucleus of the olivary complex and synapse directly with the OHCs. However, lateral efferent neurons synapse with afferent neurons which contact the IHCs. The effect of efferent neurons on the OHCs is presynaptic; meaning the neuron acts directly on the OHC. The effect of efferent neurons on the IHCs is postsynaptic, meaning that they connect directly to auditory nerve fibers (Nadol, 1983).

Inner ear physiology. When sound is transmitted from the middle ear to the cochlea a transduction process occurs in which mechanical energy is converted into electrical energy. There are two processes involved in the signal transduction: the passive process and the active process.

Sound that enters the ear canal causes the tympanic membrane to move back and forth, vibrating the ossicles. The vibration of the ossicles results in the footplate of the stapes moving in and out of the oval window. This movement results in the displacement of the fluid in the cochlea (Schubert, 1980). This causes a traveling wave to form on the basilar membrane (Bekesy, 1960). The place at which maximal displacement occurs along the basilar membrane strictly depends on the frequency of the signal. Maximal displacement occurs at the base of the cochlea for high frequency sounds and at the apex of the cochlea for low frequency sounds. The basilar membrane is narrow at the base and gradually becomes wider at the apex. As the traveling wave moves along the basilar membrane it increases in amplitude until it reaches a peak displacement and then decreases rapidly in amplitude. Movement of the basilar membrane results in movement

of the IHCs and OHCs (Gelfand, 2001). Bekesy (1948) first discovered this phenomenon known as the passive process, in cadavers.

The active process is a nonlinear function in which the cochlea makes up for the dampening of the auditory signal which occurs because the cochlea is fluid-filled. The motility of the OHCs and additional energy is added to the traveling wave resulting in greater displacement along the basilar membrane. The active process occurs at low intensity levels, approximately up to 60 dB HL, and is sensitive to damage to the OHCs (Dallos, 1988).

The movement of the basilar membrane causes the stereocilia to be displaced away from the kinocilium, the longest stereocilia on the end of the hair cell, causing excitation of the cell. When stereocilia bend, the crosslinks stretch and ion channels open. This allows positive potassium ions to flow into the cell resulting in depolarization of the cell. Simultaneously, calcium channels open and calcium flows into the cell. The influx of positive potassium ions into the hair cells starts a cascade of processes resulting in the release of neurotransmitters and generation of neural impulses being sent via the auditory nerve. This entire process changes mechanical energy into electrochemical energy by allowing the response of the hair cell to be transmitted by the auditory nerve (Hudspeth, 1985).

The areas of the inner ear, as previously discussed, can be damaged by increased levels of sound. The following section will discuss the damaging changes that occur.

Anatomy and Physiology of the Noise Damaged Auditory System

Noise induced hearing loss is a result of structural and molecular changes in the inner ear caused by excessive exposure to noise. Molecular changes occur before

structural changes are noticed. Both structural and molecular damage occurs prior to a decrease in hearing sensitivity (Wenthold, Schneider, Kim, & Dechesne, 1992). Cell death in the cochlea is caused by molecular and biochemical mechanisms. Damage to the cell results in structural changes (LePrell, 2007).

According to Wenthold et al. (1992) there are four distinct stages in which structural and molecular damage occurs. In the first stage, changes in metabolite and ion channels are exhibited. Following these molecular changes, in stage two the hair cells and the afferent pathways to the VIII cranial nerve begin to swell causing a temporary threshold shift. In the third stage of damage related to NIHL there is permanent damage to the hair cell stereocilia and reticular lamina, causing a permanent threshold shift. These changes occur because of significant biochemical changes involving proteins and lipids. Finally, in the fourth stage cell death occurs resulting in an increased permanent threshold shift.

Acoustic trauma results in cell death. There are two types of cell death: unprogrammed, also called necrotic, and programmed, known as apoptotic. Necrotic cells are swollen and usually rupture (Schweichel & Merker, 1973), whereas apoptotic cells are shrunken (Niu, Shao, & Canlon, 2003). Cell death has been studied in OHCs after exposure to damaging noise. These studies suggest that the majority of OHCs were apoptotic (Hu, Guo, Wang, Henderson, & Jiang, 2000; Yang, Henderson, Hu, & Nicotera, 2004). Niu, Shao, and Canlon (2003) found sound conditioning to result in a decrease of apoptotic cells following acoustic trauma. Acoustic trauma results in the breakdown of organelles within the cell. Sound conditioning reduces these breakdowns, protecting against cell death.

Exposure to loud sound causes over stimulation of the cochlea, weakening the ability of the cochlea to detect high frequency stimuli (Cody & Johnstone, 1980). According to Zwislocki and Cefaratti (1989), changes in the mechanical properties of the tectorial membrane and OHCs result in decreased sensitivity and tuning of the inner ear. The basilar membrane is tonotopically organized with high frequency stimulation occurring at the base and low frequency stimulation occurring at the apex. When the cochlea is over stimulated, the high frequency fibers are affected first because they are located at the base of the basilar membrane (Gelfand, 2001). The basilar membrane is highly tuned; when an acoustic stimulus is presented the shearing motion in the fluid filled cochlea causes the displacement of the cochlear partition to be reduced (Bekesy, 1960).

Hearing loss due to exposure to loud sound primarily damages the OHCs in the inner ear. Vibrations cause the movement of the tectorial membrane and basilar membrane resulting in movement of the OHCs. These vibrations differ depending on the acoustic stimuli resulting in the high frequency specificity of the cochlea. Outer hair cell damage results in a significant loss in the sensitivity and frequency specificity of the cochlea (Yost, 2000). The greatest change in hearing sensitivity often occurs one-half octave above the frequency of the noise exposure (Bilger, 1976).

Noise exposure usually results in the degeneration of OHCs. In more severe cases, such as acoustic trauma, damage to the IHCs can occur with IHCs less affected by traumatic sound than OHCs (Chen & Fetcher, 2003).

Outer hair cell damage can be reduced by exposing the cochlea to continuous low level acoustic stimuli prior to a traumatic sound (Canlon & Fransson, 1995). In this

experiment guinea pigs were conditioned using a 1 kHz tone at 81 dB SPL for 24 days followed by a traumatic exposure of a 1 kHz tone at 105 dB SPL for 72 hours. The control group, which did not receive previous noise exposure, revealed reduced or absent distortion product otoacoustic emission (DPOAE) amplitudes at all frequencies tested. The group that received previous sound conditioning revealed increased DPOAE amplitudes at all frequencies tested when compared to the control group data.

Researchers have found a partial and frequency dependent correlation between NIHL and degree of hair cell loss. Chen and Fetcher (2003) investigated OHC loss in rats with already documented NIHL. The rats were exposed to varying frequencies and intensities of noise and some animals were exposed to noise as well as hypoxic conditions. Cochlear function was assessed using compound action potential (CAP) thresholds. Outer and inner hair cell loss was measured by counting missing hair cells in perfused and fixated cochlea using Scion Image software. Hearing loss was seen at frequencies close to the frequency of the noise exposure. At some frequencies these animals exhibited NIHL; however, in some cases OHC loss was not found in corresponding regions of the cochlea. There was no hair cell loss in the low frequency region of any of the animals, even when a severe low frequency hearing loss was seen. For example, one rat exposed to 115 dB OBN and hypoxia had CAP threshold elevations of 40 to 60 dB at frequencies from 4 kHz to 8 kHz. Upon inspection of the OHCs, there was no hair cell loss in the region of the cochlea that correlated to frequencies below 8 kHz. Outer hair cell loss would have been expected with the severe level of CAP threshold elevation. There was little or no IHC loss until the threshold loss increased above 60 dB (Chen & Fetcher). This suggests that OHC loss may not be the only

contributing factor to NIHL and that hearing loss might result from present but dysfunctional hair cells.

Chen and Fetcher (2003) suspect that OHC deterioration as well as other impairments in the cochlea may also affect hearing sensitivity in animals with NIHL. In contrast, these researchers also found cochlea exhibiting OHC loss without hearing loss. This suggests that a certain amount of OHC loss will not affect hearing sensitivity and OHCs that are damaged or dysfunctional yield decreased hearing sensitivity.

Noise induced hearing loss can cause damage to the OHCs as well as damage to other parts of the cochlea. The results of Chen and Fetcher (2003) strongly suggest that factors other than loss of hair cells are involved in NIHL. These factors include damaged stereocilia and, in the case of OHCs, decoupling from the tectorial membrane. Both of the suggested mechanisms may result in permanent hearing loss or may be reversed after a prolonged period of time resulting in hearing restoration. It is possible to expect varying impacts on sound utilization depending on the dysfunction of outer and inner hair cells. Damage of IHCs should have a larger impact on speech discrimination than damage of OHCs.

The survival and functionality of hair cells are important. The damage caused by excessive exposures to noise is not only a result of the mechanical destruction of the hair cells but also of the blood flow to the inner ear, and increased metabolic activity (Lim & Melnick, 1971). Overstimulation of the cochlea increases metabolic activity resulting in the increase of free radicals (LePrell, 2007). Therefore, measurements of otoacoustic emissions can be a crucial part of the evaluation of the effect of noise exposure, as they assess functional properties of OHCs.

Measurements of Hearing

Otoacoustic emissions. In a healthy cochlea, the movement of OHCs create sound vibrations which are emitted into the ear canal (Kemp, 2002). These low level sounds are known as otoacoustic emissions (OAEs) and can be measured by the placement of a microphone in the ear canal. Otoacoustic emissions can be measured approximately 5 ms after the onset of the stimulus (Gelfand, 2001). There are two main types of OAEs that are used clinically: transient OAEs and distortion product OAEs. Distortion product OAEs have been used to study the damaging effects of noise as it offers possibilities of monitoring higher frequencies and will be further discussed.

Distortion product OAEs are distortions formed in the cochlea in response to two pure tone stimuli presented at the same time. These responses are referred to as distortions because they are produced in the cochlea as a tonal signal at a frequency which is not presented in the two pure tone stimuli. The intensity of the lower frequency pure tone is known as L_1 and the intensity of the higher frequency pure tone is known as L_2. The $2F_1$-F_2 frequency is the most frequently used distortion product (Lonsbury-Martin & Martin, 2002).

The amount of damage caused by exposure to noise can be determined by measuring OAEs in the ear. Otoacoustic emission testing measures the integrity of the OHCs and therefore is an important indicator of OHC loss following exposure to noise. By measuring DPOAEs before and after sound exposure, researchers are able to determine the amount of OHC loss caused by the noise exposure (Lonsbury-Martin & Martin, 2002). Distortion product OAEs are an objective measure of OHC function and are used in both humans and animals (Shera & Guinan, 1999).

Auditory brainstem response. The auditory brainstem response (ABR) is an auditory evoked transient potential. Transient indicates that a single stimulus is presented resulting in a single response. An external stimulus is presented, e.g., click stimulus, and the electrical responses of the nervous system are measured. The ABR averages the neural responses which reduces components that are not related to the electrical responses to sound. This evoked response measures the activity generated by the VIII cranial nerve and other areas within the brainstem that are responding to the external stimuli (Hood, 1998).

There are two main clinical applications for the ABR: (a) the evaluation of hearing threshold and (b) the detection of neurological abnormalities of the eighth nerve and auditory pathways (Hood, 1998). The former is used to determine the effects of damaging noise exposure in animals.

Paradigms that Toughen the Cochlea

It is speculated that toughening the auditory system through prior low level noise exposure may prove useful in occupational settings where hearing protection devices are not available. Professionals in these occupations include firefighters, police officers, and military personnel. Sound conditioning or auditory toughening may also provide additional protection in cases where traditional hearing protection devices, such as ear plugs or ear muffs, are already available (Berger & Lindgren, 1992).

Sound conditioning of the auditory system is one way to reduce the effects of noise exposure on hearing. This mechanism should allow the cochlea to be exposed to increased levels of noise without experiencing significant PTS and damage to the OHCs. Ears that have been conditioned by low level noise exposures are less sensitive to

additional traumatic sound exposures. Possible protective mechanisms in the cochlea are not completely understood at this time. This resistance to damaging noise is known as sound conditioning or auditory toughening and has been studied in a variety of animals (Canlon, 2002).

Sound conditioning and auditory toughening are two different methods used to make the auditory system less sensitive to traumatic sound exposures. Both methods result in the resistance of the cochlea to a traumatic noise. These two terms are often used interchangeably; however, sound conditioning is a continuous low level non-damaging noise presented prior to a traumatic exposure; whereas, auditory toughening is an interrupted acoustic stimulus presented prior to a traumatic sound exposure. The stimulus is presented for a given period of time, ranging anywhere between 6 to 12 hours, and then turned off for a given period of time, ranging anywhere between 12 to 18 hours (Canlon, 2002). The interrupted acoustic stimuli cause a temporary threshold shift in the first few days of exposure (Canlon; Niu & Canlon, 2002).

Sound conditioning effects have been observed in guinea pigs (Canlon & Fransson, 1995), gerbils (Ryan, Bennett, Woolf, & Axelsson, 1994), and mice (Yoshida & Liberman, 2000). Auditory toughening has been studied in a variety of animals including chinchillas (Campo et al., 1991; McFadden, Henderson, & Shen, 1997; Subramaniam, Henderson, Campo & Spongr, 1992; Subramaniam, Henderson, & Spongr, 1993b) and guinea pigs (Subramaniam, Campo, & Henderson, 1991a, 1991b; Subramaniam et al., 1992; Campo et al). The term sound conditioning is often used to describe both methods. For the purpose of this paper sound conditioning will be used to

describe a continuous acoustic stimulus and auditory toughening will be used to describe an interrupted acoustic stimulus.

Sound conditioning. Sound conditioning has been found to reduce permanent threshold shifts when animals are exposed to a traumatic sound (Canlon et al., 1988; Canlon & Fransson, 1995; Canlon & Fransson, 1998; Yoshida & Liberman, 2000). The literature regarding sound conditioning is extensive. Many studies using a variety of stimulus parameters have yielded similar results.

Threshold shifts are reduced significantly following sound conditioning. Studies have shown that over an extended period of time, the auditory system completely recovers and no threshold shift is indicated if initial damage is limited. This phenomenon was evident 8 weeks after a traumatic sound exposure in guinea pigs. After 8 weeks, the conditioned group showed complete recovery, whereas the non-conditioned group had threshold shifts between 14 dB and 35 dB (Canlon et al., 1988).

There are a variety of animals used to study the effects of sound conditioning as well as varying durations of conditioning. As outlined in Table 2, mice (Yoshida & Liberman, 2000) and guinea pigs (Canlon & Fransson, 1998; Canlon et al., 1988) are primarily used for these studies. At this time there is a lack of literature pertaining to sound conditioning in rats.

Table 2. Animals Used and Duration of Sound Conditioning

Animals Used	Duration of sound conditioning
Mice	15 minutes
	7 days (24 hr/day)
Guinea Pigs	13 days (24 hr/day)
	24 days (24 hr/day)

Sound conditioning parameters include duration, frequency /spectrum, and intensity of the acoustic stimulus. The amount of time it takes for the cochlea to develop a protective effect is still unknown and varies among studies. The length of the sound conditioning exposure ranges from 15 minutes (Yoshida & Liberman, 2000) to 24 days (Canlon & Fransson, 1998; Canlon et al., 1988) as shown in Table 2. Unlike auditory toughening, animals are exposed to the conditioning stimulus 24 hours per day.

The frequency and intensity of the sound conditioning stimulus varies across studies as well. Table 3 displays these differences. Canlon and Fransson (1998) used pure tone conditioning stimuli of 6.3 kHz and Canlon et al. (1988) used pure tone conditioning stimuli of 1 kHz when conditioning guinea pigs. Yoshida and Liberman (2000) used OBN (8-16 kHz) when conditioning mice. The intensity of the conditioning stimuli also varied between 78 dB SPL (Canlon & Fransson) and 89 dB SPL (Yoshida & Liberman). As with differences between other stimulus parameters, differences in stimulus intensities and frequencies across studies make it difficult to interpret and compare the results.

Table 3. Frequency and Intensity Parameters of Sound Conditioning

Frequency	Intensity
1 kHz pure tone	81 dB SPL
6.3 kHz pure tone	78 dB SPL
8 kHz-16 kHz OBN	81 dB SPL
	89 dB SPL

The amount of time between the end of sound conditioning and a traumatic sound exposure, known as the rest period, affects the degree of change in threshold shifts. Rest periods range from 2 hours to 7 days (Canlon & Fransson, 1998) and in one study the rest period was not specified (Canlon et al., 1988). The varying results using different periods of rest indicate that the conditioning effect may wear off after a given period of time. Following 15 minutes of sound conditioning, Yoshida and Liberman (2000) found a significant reduction in threshold shifts after a rest period of 24 hours, whereas a rest period of 48 hours did not reveal significant changes in threshold shifts between the control group and experimental group.

Canlon and Fransson (1998) studied sound conditioning effects in guinea pigs. This study consisted of four experimental groups and one control group. The experimental groups were conditioned for either 13 days or 24 days. The animals conditioned for 13 days received a traumatic exposure, either 2 hours or 7 days after conditioning. The animals conditioned for 24 days received a traumatic exposure, either 2 hours or 7 days after conditioning. Animals exposed to the traumatic sound 2 hours following conditioning exhibited greater threshold shifts than animals exposed to the

traumatic sound 7 days after conditioning, regardless of the duration of sound conditioning. Significant reductions in DPOAE amplitudes were also seen in the groups conditioned for 13 days and 24 days with a 2 hour rest period compared to the control group. Protection was observed in animals that were given a 7 day rest period; however, protection was reduced compared to animals given a 2 hour rest period.

The frequency, intensity, and duration of traumatic sound exposures are variable. Table 4 outlines these parameters. In each of these studies the same stimuli was used for sound conditioning and traumatic sound exposures; however, different intensities were used. The stimuli range from a 1 kHz or 6.3 kHz pure tone to an OBN between 8 kHz and 16 kHz. The intensities range from 100 dB SPL (Canlon & Fransson, 1998; Yoshida & Liberman, 2000) to 105 dB SPL (Canlon et al., 1988) with durations between 2 hours (Yoshida & Liberman) and 72 hours (Canlon et al.).

Table 4. Traumatic Sound Parameters

Frequency	Intensity	Duration
1 kHz pure tone	105 dB SPL	72 hours
6.3 kHz pure tone	100 dB SPL	24 hours
8 kHz-16 kHz OBN	100 dB SPL	2 hours

Yoshida and Liberman (2000) found that sound conditioning for as little as 15 minutes and as long as 7 days can reduce permanent threshold shifts from a traumatic sound exposure (OBN at 100 dB SPL for 2 hours). More protection was afforded when there was a 24 hour rest period, resulting in a PTS less than 20 dB. The conditioned group, given a 48 hour rest period, experienced no protection from sound conditioning.

The thresholds of the conditioned group were similar to the thresholds of the control group.

Auditory brainstem responses revealed significantly lower threshold shifts for the conditioned group when compared to the group only exposed to the traumatic noise. Thresholds of the control group shifted 33 to 53 dB across all frequencies, while thresholds of the experimental group only shifted 8 to 40 dB (Canlon et al., 1988).

Canlon and Fransson (1998) studied the protective effect in guinea pigs that were conditioned for either 13 days or 24 days when the traumatic sound exposure occurred 2 hours following the conditioning stimulus. There was no difference in threshold shifts between the two sound conditioned groups. Following a 1 week rest period, protection was noticed but not as much protection as after a 2 hour rest period. This suggests that after a certain amount of time the conditioning effect may wear off and protection from a traumatic exposure may not be seen.

It is apparent that results of sound conditioning following a traumatic exposure are dependent on a variety of factors including stimulus intensity, stimulus frequency, and the amount of rest between sound conditioning and traumatic sound exposure. Since these parameters vary greatly across studies, more research is warranted in this area. A variety of test parameters are also seen in the auditory toughening literature, as described below.

Auditory toughening. Auditory toughening, the use of an interrupted sound, is more applicable in real life situations compared to the continuous stimulus used for sound conditioning. Interrupted stimuli more closely mimic industrial and recreational exposures to noise than continuous stimuli.

The auditory system gradually becomes resistant to noise over a period of time; this is achieved by exposing the cochlea to an interrupted acoustic stimuli. Several researchers have shown that a subsequent exposure to the same stimulus at a higher intensity level often results in a smaller PTS as compared to a control group only exposed to the high intensity stimuli (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1992; Subramaniam et al., 1993b). As with sound conditioning, a variety of auditory toughening stimulus parameters have been used resulting in reduced PTS following a traumatic noise. The results from these studies are outlined below.

Auditory toughening parameters include duration, frequency, and intensity of the stimulus. The number of days that the toughening stimulus is presented varies; however, most studies expose the animals to the stimulus for 6 hours per day. The average number of days used to toughen the auditory system is 10 days; however, Subramaniam et al. (1993b) used as little as 2 days for 6 hours per day, one exposure on the first day and the second exposure on the tenth day. These investigators also used 20 days for 6 hours per day. These parameters are shown in Table 5.

Table 5. Animals Used and Duration of Auditory Toughening

Animals Used	Duration
Chinchillas	6 hr/day 1st and 10th day only
	6 hr/day 10 days
	6 hr/day 20 days

Another variable is the frequency and intensity at which the toughening stimulus is presented as described in Table 6. Many researchers used a 500 Hz OBN stimulus

presented at either 90 or 95 dB SPL (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1993b). In contrast, Subramaniam et al. (1992) used a toughening stimulus of 4 kHz OBN at an intensity of 85 dB SPL.

Table 6. Frequency and Intensity Parameters of Auditory Toughening Stimulus

Frequency	Intensity
500 Hz OBN	90 dB SPL
	95 dB SPL
4 kHz OBN	85 dB SPL

Auditory toughening often results in a TTS during the first few days of toughening (Subramaniam et al., 1992), whereas a threshold shift is not usually exhibited during sound conditioning. Subramaniam et al. (1992) recognized a 10 dB threshold shift at 2 kHz on the first and second day of toughening; this threshold shift decreased to 5 dB or less by the seventh day of toughening. A decrease in threshold shifts over the 10 days of toughening was also experienced for other frequencies. A significant threshold shift occurred at all frequencies after 10 days of toughening at an intensity of 95 dB SPL (McFadden et al., 1997). However, significant threshold shifts were only measured at 500 Hz to 4 kHz after 10 days of toughening at an intensity of 90 dB SPL. Both of these groups used a 500 Hz OBN toughening stimulus. After a 5 day rest period the thresholds returned to normal.

Research suggests that there is not a significant difference in hearing thresholds measured before and 5 days after the auditory toughening exposure (Campo et al., 1991; Hamernik & Ahroon, 1999; McFadden, et al., 1997). Subramaniam et al. (1993b) found

varying results between thresholds before toughening and 5 days after toughening. These differences were dependent on the duration of the toughening exposure. The hearing thresholds of chinchillas exposed to the toughening stimulus for 10 days were the same as pre-exposure thresholds. However, chinchillas exposed to the toughening stimulus only on the first and tenth day and for 20 days had threshold shifts of 5 to 10 dB and 10 to 15 dB respectively.

Similar to differences in the length of the toughening exposure, differences in the rest period also affect the degree of threshold shifts. Subramaniam et al. (1992) studied the hearing thresholds of chinchillas 18 hours and 5 days after auditory toughening. Animals that had an 18 hour recovery period exhibited a 10 to 15 dB threshold shift, whereas the animals with a 5 day recovery period did not have threshold shifts when compared to pre-exposure thresholds. McFadden et al. (1997) found no significant differences in threshold shifts in chinchillas given a 30 day or 60 day recovery period between toughening and traumatic exposures.

A variety of stimulus parameters have been used for the traumatic sound stimuli. These include varying the frequency, intensity, and duration of the stimuli. These parameters are shown in Table 7. Campo et al. (1991), McFadden et al. (1997), and Subramaniam et al. (1993b) presented a traumatic sound stimulus of 500 Hz OBN at 106 dB SPL for 48 hours, whereas Subramaniam et al. (1992) presented a 4 kHz OBN stimulus at 100 dB SPL for 48 hours. The frequency of the interrupted noise and the frequency of the traumatic sound exposure were the same (Campo et al; McFadden et al.; Subramaniam et al.; Subramaniam et al.).

Table 7. Traumatic Sound Parameters

Frequency	Intensity	Duration
500 Hz OBN	106 dB SPL	48 hours
4 kHz OBN	100 dB SPL	

The greatest threshold shifts occurred immediately following the traumatic sound exposure. In most studies, substantial recovery was observed 24 hours post traumatic exposure and continued to recover gradually thereafter (McFadden et al., 1997; Subramaniam et al., 1993b). McFadden et al. found that 24 hours after the traumatic exposure average thresholds recovered by roughly 23 to 31 dB and after 5 days of recovery thresholds recovered an additional 13 to 31 dB.

Evoked response recordings suggest variable results regarding auditory toughening with respect to frequency, duration, and intensity. An auditory toughening stimulus of 500 Hz OBN resulted in smaller threshold shifts between 500 Hz and 4 kHz in the toughened group than in the group only exposed to the traumatic sound (Campo et al., 1991). These thresholds were approximately 20 to 30 dB better and did not recover more than 5 dB 5 days after the traumatic exposure. A protective effect was exhibited between the frequencies of 500 Hz and 4 kHz when exposed to a 500 Hz toughening and traumatic exposure stimulus.

Subramaniam et al. (1992) studied chinchillas that were introduced to a traumatic sound either 18 hours or 5 days after the toughening exposure. Interestingly, the control group which did not have any toughening exposure had a smaller threshold shift than the group given 5 days to recover after toughening. The 5 day recovery group had the

greatest permanent threshold shift at 4 kHz and 5.6 kHz, whereas the 18 hour recovery group had the least permanent threshold shift at those two frequencies.

McFadden et al. (1997) studied chinchillas exposed to a 500 Hz OBN stimulus at 90 or 95 dB SPL and reported these animals experienced significantly less threshold shifts at 1 kHz, 8 kHz and 16 kHz. In contrast, the control group experienced significant threshold shifts at all frequencies tested.

All of the studies discussed above used a toughening duration of 6 hours a day for 10 days; however, Subramaniam et al. (1993b) also used two additional toughening durations. One exposure was for 6 hours a day for 20 days and the other exposure was for 6 hours a day only on the first and tenth day. There was no significant difference between groups that incurred auditory toughening for 10 and 20 days when compared to each other. Threshold shifts were significantly reduced in all groups compared to the control group.

McFadden et al. (1997) used toughening intensities of 90 and 95 dB SPL. Similarly, there was no significant difference between animals exposed to a 90 dB toughening stimulus and animals exposed to a 95 dB toughening stimulus.

Histological examination revealed no significant difference in OHC loss between control animals and toughened animals (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1992; Subramaniam et al., 1993b). Interestingly, Campo et al. reported that hair cell damage in control animals occurred in the low to mid frequencies and hair cell damage in the toughened animals occurred approximately one octave above the toughening exposure. The reason for this is unknown. Inner hair cell loss was calculated in three of the four studies reviewed. McFadden et al. and Subramaniam et al.

(1992) found no significant difference in IHC loss between the control animals and the toughened animals. Subramaniam et al. (1993b) found minimal IHC loss in all groups.

Although the studies previously discussed used the same stimulus for the toughening and traumatic exposure, other studies have used different stimuli. Subramaniam, Henderson, & Spongr (1993a) used a low frequency interrupted exposure and a high frequency traumatic exposure. Results revealed a greater threshold shift in the experimental group compared to the group only exposed to the high frequency traumatic noise. This suggests that toughening the chinchillas' cochlea using one frequency will not protect it from a traumatic sound of a different frequency.

If the traumatic sound is too loud, a toughening effect may not be observed. Hamernik and Ahroon (1999) used an interrupted noise exposure of 500 Hz at 95 dB SPL for 6 hours per day for 10 days. Five days later the chinchillas were exposed to a traumatic sound exposure of 127 dB SPL narrow band impact noise centered at 1 kHz, 24 hours a day for 5 days. The interrupted noise exposure did not yield protection from the traumatic sound exposure and there was no statistical difference in OHC loss between the control and experimental groups (Hamernik & Ahroon). This lack of protection suggests either the traumatic exposure intensity was too loud or that different stimuli used for the toughening and traumatic exposure did not exhibit protection.

The variability of stimulus parameters between studies makes it difficult to compare the results. Auditory toughening has been measured using interrupted noise exposures ranging from 85 dB SPL to 95 dB SPL and traumatic sound exposures ranging from 100 dB SPL to 127 dB SPL. Also, the amount of time between the interrupted noise exposure and the traumatic sound exposure varied from 5 days to 60 days (Canlon

et al. 1988; Canlon & Fransson, 1998; Yoshida & Liberman, 2000). The amount of time that the protective effect lasts is still unknown.

Chinchillas are often used when studying the effect of auditory toughening on the cochlea. Limited research has been conducted studying the effect of auditory toughening on the rat cochlea.

More research is necessary both in the areas of sound conditioning and auditory toughening, particularly to define the level of protection afforded by the cochlea. Parameters such as the intensity, frequency, and duration of the conditioning/toughening stimuli; the rest period between conditioning/toughening and the traumatic exposure; and the intensity, frequency, and duration of the traumatic sound need to be evaluated to determine which parameters yield the best protective results.

Purpose of Study

Exposure to noise can cause cochlear damage. There is a considerable need for protection against noise induced hearing loss. Hearing protection devices (i.e. earplugs and earmuffs) provide protection against damaging sounds; however, they cannot be used in every situation and negative side effects include problems with communication. Other protective strategies, such as sound conditioning and auditory toughening, have also been found to provide protection by reducing the effects of a traumatic sound on the auditory system. Auditory toughening, which uses an interrupted stimulus, is more applicable in real life situations compared to a continuous stimulus used with sound conditioning. It is thought that auditory toughening may be an effective protective strategy particularly for individuals who are in situations where hearing protection devices are not practical or feasible such as the military, police force, and fire department. A variety of parameters

have been studied on animals in regards to auditory toughening and its effects on hearing; however, the ranges of parameters which offer protection are still not clearly determined. This study is designed to further explore the toughening effects on the cochlea, more specifically different stimulus intensities in which the protective effect is observed.

For the present study it can be hypothesized that the experimental groups will have less hearing damage as measured by ABR and DPOAE than the control group not exposed to the toughened stimuli. A null hypothesis would state that there is no difference in threshold shifts between the two experimental groups and the control group.

CHAPTER 3

METHODS

Subjects

Twenty one pigmented male Long Evans rats (175-200 g) were used to delineate the protective effects of auditory toughening on noise induced hearing loss. The animals were housed in individual cages in a room kept at 72 degree Fahrenheit with 12 hours of lighting. The care of the animals in this study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland Medical School. All animals were allowed free access to food and water. The rats were divided into three groups: control group (kept in normal laboratory ambient background sound) and two experimental groups exposed to two different levels of auditory toughening sound.

Experimental Paradigm

Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emission (DPOAE) measurements were performed at the beginning of the experiment (Stage 1), after auditory toughening or waiting period for control group (Stage 2), immediately after exposure to the traumatic sound (Stage 3), and 10 to 12 days allowed for recovery (Stage 4) (Table 8).

Table 8. Stages of Experiment

Stage	Description of Stage
1	Baseline
2	After toughening or ambient noise
3	After traumatic sound exposure
4	After 10-12 days allowed for recovery

ABR Measurements

Tone burst ABR thresholds were measured using BioSig Version 2.0 equipment to estimate audiometric thresholds. Responses were measured using subcutaneous electrodes placed at the vertex (active), left mastoid (reference), and right front limb (ground). Testing was completed in a sound proof box. The air-conducted stimulus was presented through an insert earphone inserted in the left ear canal. A one-channel ABR recording was measured at the following frequencies: 6.3k, 8k, 10k, 12.5k, 16k and 20k Hz. The presentation of a linear-gated tone was 21.0 pips per second and responses were recorded and averaged to 1024 sweeps per condition. The stimulus intensity was decreased by 5 dB starting at a relative point of zero of the attenuator until threshold was established. The intensity of the relative starting point was frequency dependent. Table 9 illustrates the intensity in dBA and recalculated to dB SPL that was equivalent to zero dB attenuation at each frequency. The ABR response was replicated for all responses within 20 dB of threshold. Threshold was considered the lowest intensity in which wave V of the ABR could be replicated. Wave V latencies were compared to normative data previously obtained in the lab.

Table 9. ABR Intensity Equivalents for Zero Setting of the Attenuator

Frequency	dBA	dB SPL
6.3 kHz	101	101.12
8 kHz	70	71.15
10 kHz	76	79.49
12.5 kHz	69.5	73.75
16 kHz	69	75.71
20 kHz	51.5	60.85

DPOAE Measurements

Distortion product OAEs were recorded using the Starkey Laboratories DP 2000 Measurement System. The $2f_1$-f_2 DPOAEs were measured at six points per octave between the frequencies of 6k Hz and 16k Hz using L_1/L_2 intensities of 65/55 dB SPL. The f2/f1 ratio was 1.20. These measurements were repeated twice and an emission was considered present if it was at least 3 dB above the noise floor at that frequency.

Rats Preparation

Animals were anesthetized with an intraperitoneal injection of Nembutal (50mg/kg) for ABR and DPOAE measurements as well as traumatic sound exposures. During the measurements if the animals were not sufficiently anesthetized (judged by paw withdrawal reflex), an additional dosage of Nembutal (1/3–1/2 of the original dose) was given. During anesthesia the body temperature was monitored with a rectal thermometer and maintained at 37.5 degrees Celsius using the Harvard Homeothermic Blanket Control Unit.

Stage 1 - Baseline

Prior to the exposure of the toughening sound the baseline of ABR thresholds and DPOAE amplitudes were collected for the control group (n=6) and two experimental groups (n=8 and n=7) of rats. Due to extenuating circumstances in the lab, the control animals received subcutaneous injections of saline, 5 mg/kg once per day for 10 days prior to the traumatic exposure.

Stage 2 - After Toughening or Ambient Noise

Control rats were exposed to ambient background noise of 47.5 dBA (48.65 dB SPL at an octave band centered at 8 kHz). The conversion of dBA to dB SPL intensities are displayed in Table 10. The experimental groups were exposed to the auditory toughening stimuli, a broad band noise (BBN) signal of either 60 dBA or 72 dBA (61.15 dB SPL and 73.15 dB SPL levels for an octave band filter centered at 8 kHz - see Table 10) measured close to their designated cages and presented during light cycle, 12 hours per day for 5 days. ABR thresholds and DPOAE amplitudes were measured immediately after the toughening period.

Table 10. dBA to dB SPL Conversion Table

Filters (Hz)	47.5 dBA	60 dBA	72 dBA	Correction factor
6300	47.62 dB SPL	60.12 dB SPL	72.12 dB SPL	+0.12 dB
8000	48.65 dB SPL	61.15 dB SPL	73.15 dB SPL	+1.15dB
10000	49.99 dB SPL	62.49 dB SPL	74.49 dB SPL	+2.49 dB
12500	51.75 dB SPL	64.25 dB SPL	76.25 dB SPL	+4.25 dB
16000	54.21 dB SPL	66.71 dB SPL	78.71 dB SPL	+6.71 dB
20000	56.85 dB SPL	69.35 dB SPL	81.35 dB SPL	+9.35 dB

Stage 3 - After Traumatic Sound Exposure

All animals were exposed to a 7.8 kHz pure tone stimulus at 110 dB SPL for 20 minutes presented unilaterally to the left ear utilizing a calibrated closed system 48 hours after completing the auditory toughening. ABR thresholds and DPOAE amplitudes were measured immediately after the traumatic sound exposure.

Stage 4 - After 10 to 12 Days Allowed for Recovery

The animals were allowed to recover for 10 to 12 days after the traumatic sound exposure in their home cages in the presence of ambient sound and then again ABR thresholds and DPOAE amplitudes were measured.

Statistical Analysis

Descriptive statistics including standard errors of mean (SEM), mean, and distribution were calculated for each parameter measured. Auditory brainstem responses and DPOAE measurements over all frequencies were analyzed using analysis of variance with repeated measures (ANOVA). Contrast analysis statistics were calculated for all means of ABR and DPOAE measurements. T tests for independent and paired data were subsequently performed, when indicated. Correlation analysis was performed as well.

CHAPTER 4

RESULTS

ABR thresholds and DPOAE amplitudes were evaluated in three groups of rats (control group, and two experimental groups exposed to 60 dBA or 72 dBA broadband toughening sound) at four experimental stages (baseline, after auditory toughening, after traumatic sound exposure, and after 10 to12 days of recovery). All results are presented for each group separately in Figures 1 through 3 for ABR and Figures 4 through 6 for DPOAE.

An analysis of variance (ANOVA) with repeated measures was performed comparing the ABR thresholds for all frequencies tested between the three groups and all experimental stages (Table 11). There was a significant difference in ABR measurements ($p<0.000$) for stage variable. Thus, the average thresholds of ABR changed in a significant manner for different stages of the experiment. A statistically significant difference was observed for frequency ($p<0.000$) and group variables ($p<0.01$) as well. Significance of frequency variable was expected as it has been documented that the thresholds of rat's hearing change significantly in the analyzed frequency range. Moreover, significance of group variable showed that the sound level used for auditory toughening affected ABR measurements. The interaction between the three groups and the stages that were tested was significant ($p<0.000$). This indicates that changes in ABR thresholds assessed during the four stages of the experiment were different for the three groups.

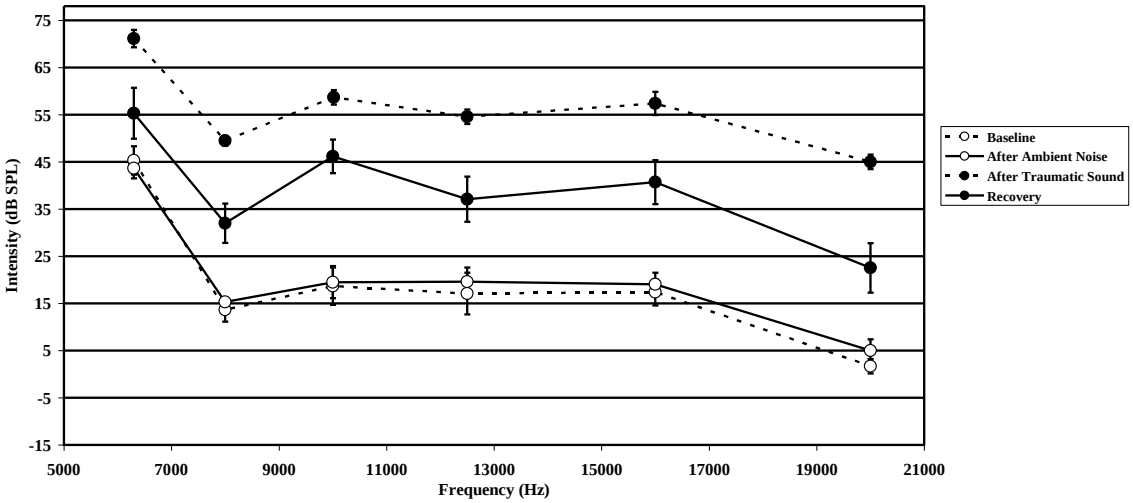

Figure 1. ABR thresholds for control group at the four experimental stages.

The x-axis represents frequency measured in Hz and the y-axis represents sound intensity easured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after ambient noise and recovery (stages 2 and 4). The vertical lines depict standard error of mean (SEM) values for this figure and subsequent figures.

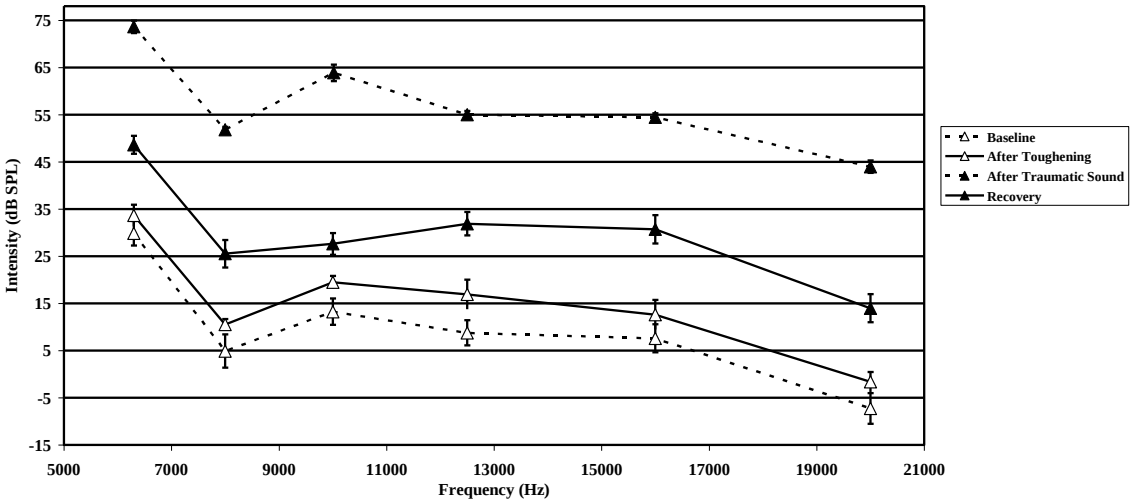

Figure 2. ABR thresholds for 60 dBA group at the four experimental stages. The x-axis represents frequency measured in Hz and the y-axis represents sound intensity measured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after toughening and recovery (stages 2 and 4).

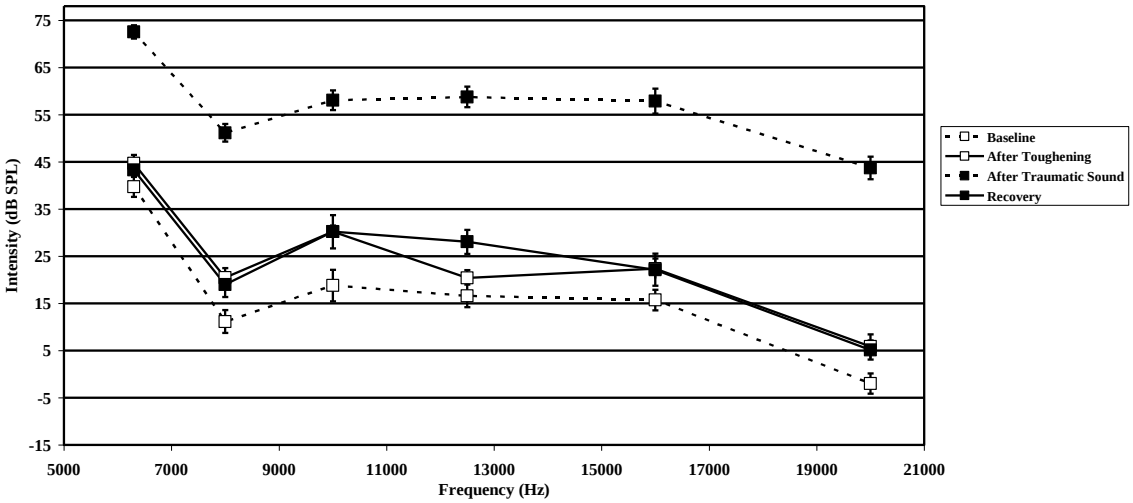

Figure 3. ABR thresholds for 72 dBA group at the four experimental stages. The x-axis represents frequency measured in Hz and the y-axis represents sound intensity measured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after toughening and recovery (stages 2 and 4).

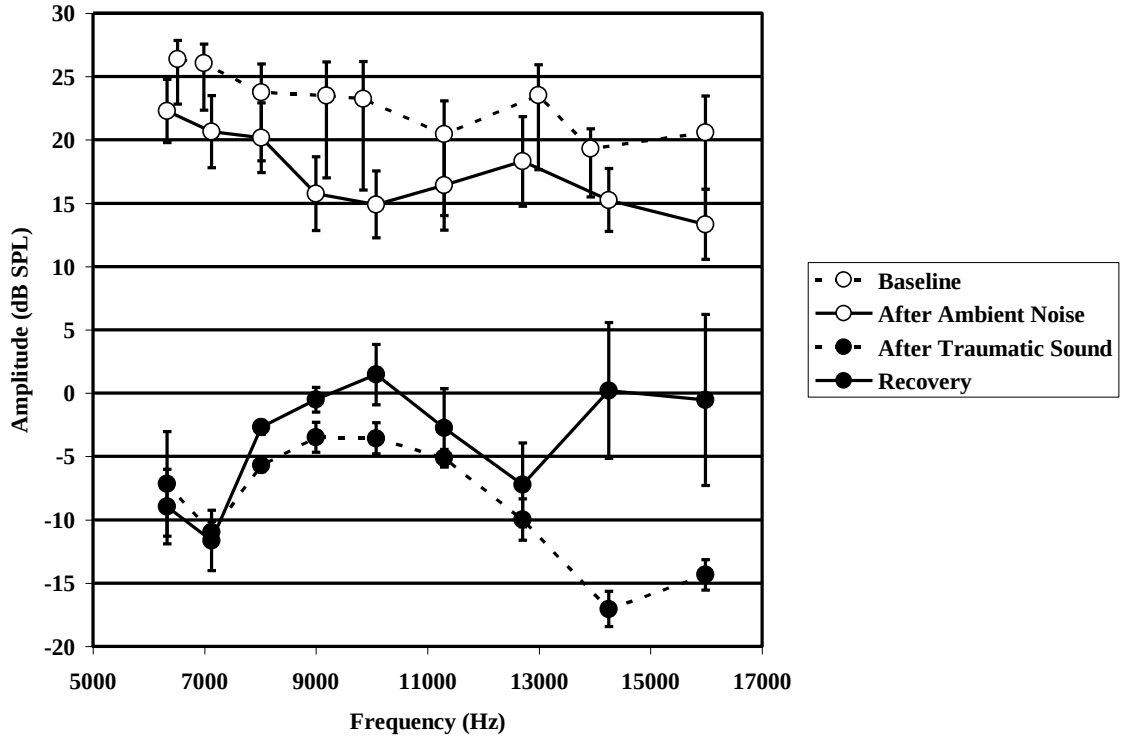

Figure 4. DPOAE amplitudes for control group at the four experimental stages. The x-axis represents frequency measured in Hz and the y-axis represents amplitude measured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after toughening and recovery (stages 2 and 4).

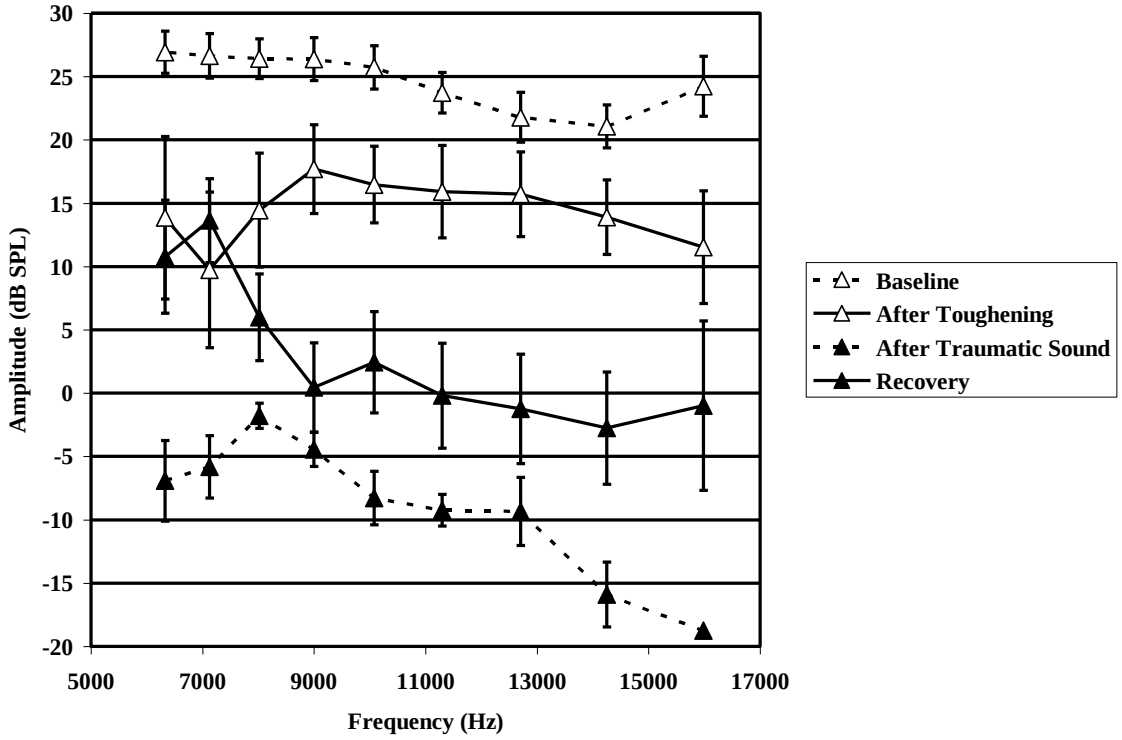

Figure 5. DPOAE amplitudes for 60 dBA group at the four experimental stages. The x-axis represents frequency measured in Hz and the y-axis represents amplitude measured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after toughening and recovery (stages 2 and 4).

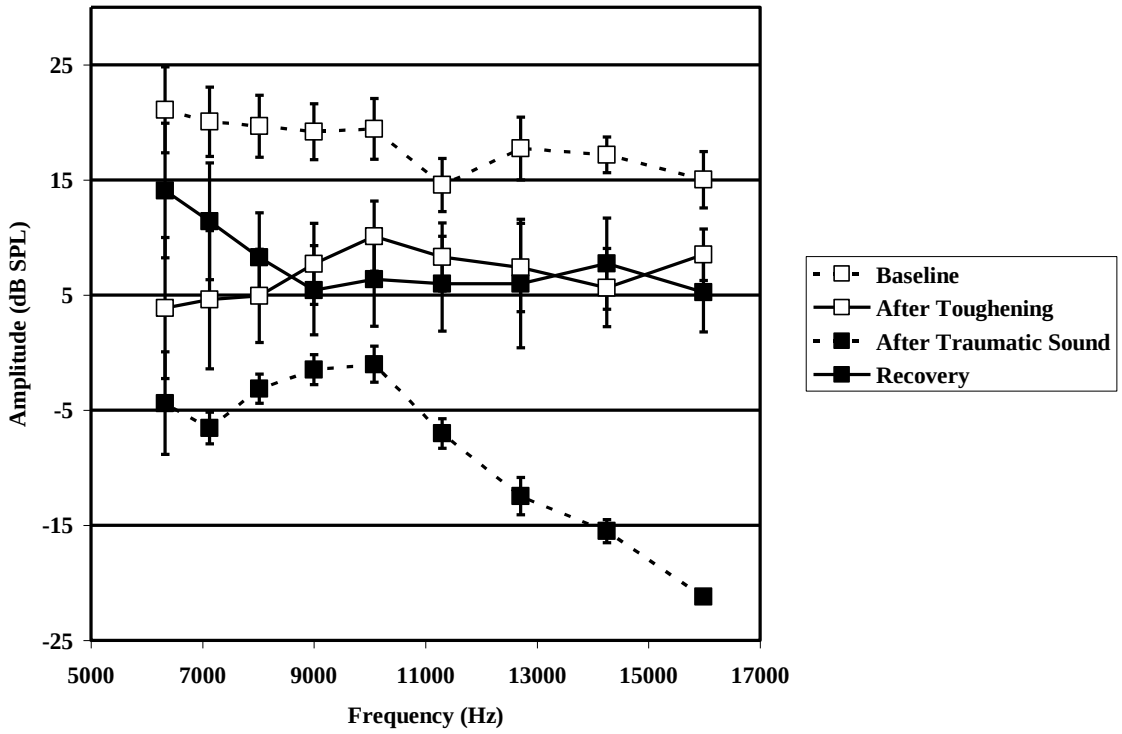

Figure 6. DPOAE amplitudes for 72 dBA group at the four experimental stages. The x-axis represents frequency measured in Hz and the y-axis represents amplitude measured in dB SPL. Open symbols represent thresholds prior to traumatic sound exposure and closed symbols represent thresholds after traumatic sound exposure. Dashed lines represent measurements taken at baseline and after the traumatic sound exposure (stages 1 and 3) and solid lines represent measurements taken after toughening and recovery (stages 2 and 4).

Table 11. ANOVA with Repeated Measures for ABR for All Frequencies

Variable	F	df	Significance
Stage	522.52	3/15	0.000
Frequency	411.81	5/13	0.000
Group	6.91	2/17	0.006
Stage*Frequency	1.29	15/3	NS
Stage*Group	6.61	6/32	0.000
Frequency*Group	0.73	10/28	NS
Stage*Frequency* Group	0.89	30/18	NS

Notably, for ABR measurements all interactions with frequency were not significant. This indicates that changes in ABR were not frequency dependent both for groups and stages. Therefore subsequent analyses were conducted by collapsing frequencies and using the mean over frequency ABR thresholds for each animal. On the other hand, highly significant factors other than frequency and their interactions warrant subsequent analyses described below.

The averaged over frequency ABR thresholds are presented in Figure 7. Visual inspection of the results suggested several observations that were statistically evaluated. First, animals showed an increased threshold of hearing after auditory toughening. Second, exposure to a traumatic sound caused substantial increase of ABR with thresholds which appear to be similar for all groups. Third, there was clear recovery 10 to 12 days after sound exposure with indication of its extent dependent on the background sound level or toughening level. Moreover, it appears that the experimental groups differed at the baseline and after toughening stages in the same manner, i.e., 62 dBA group showed consistently lower thresholds than the other groups. To investigate the validity of these observations the following set of ANOVAs was performed.

An ANOVA with repeated measures was performed on data averaged over frequencies comparing the mean ABR thresholds between the three groups at the four stages (Table 12). There was a highly statistically significant difference in ABR thresholds for the four stages during the experiment ($p<0.000$) as well as a significant interaction of stage and group variables ($p<0.000$). These findings allowed subsequent ANOVAs and t-tests to asses the differences between specific means to be performed.

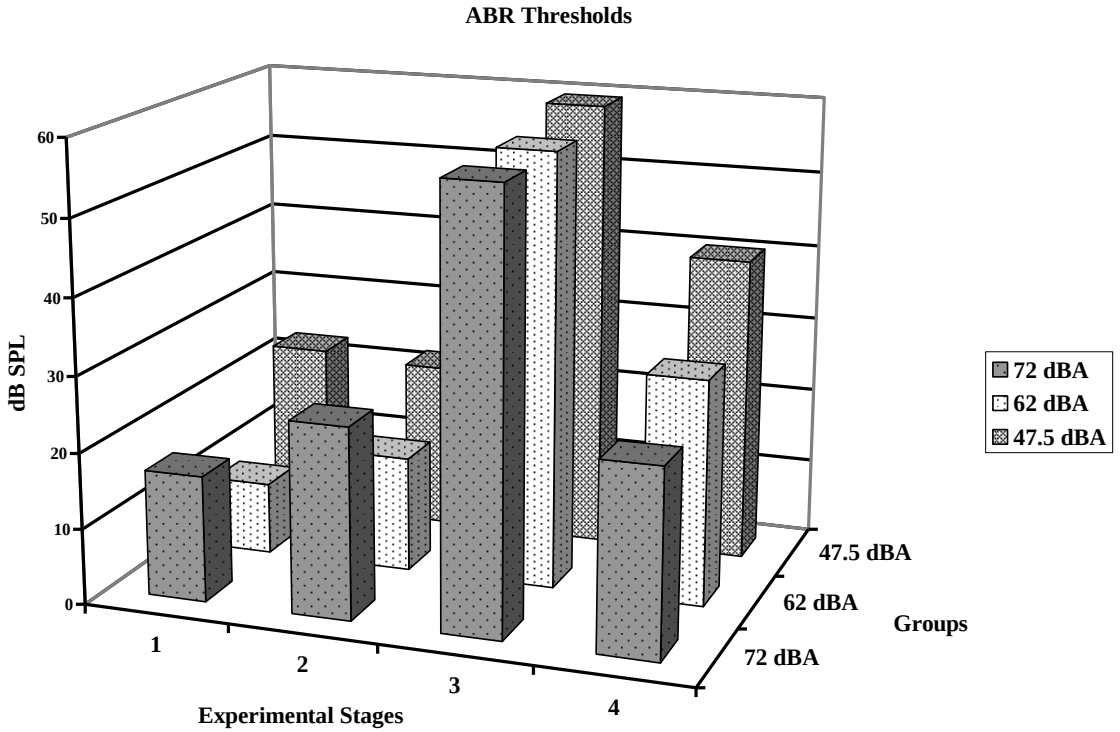

Figure 7. Mean over frequency ABR thresholds for the three groups at each stage of the experiment. The four experimental stages are shown on the x-axis and the three groups are shown on the y-axis. The mean over frequency ABR thresholds are measured in dB SPL shown on the z-axis.

Table 12. ANOVA with Repeated Measures for Mean Over Frequency ABR Data

Variable	F	df	Significance
Stage	461.50	3/16	0.000
Stage*Group	7.01	6/34	0.000

Analysis of variance with repetition was performed for each group separately (Table 13). Stage variable was significant within all three groups. These findings allowed a set of paired sample t-tests to be performed. The paired sample t-tests determined the significance of the difference between the means for specific stages analyzed separately within each group. Six comparisons were made for each group. The Bonferroni Correction method was used to divide the probability of significance provided by the binomial coefficient in the ANOVA. (e.g., to have $p<0.05$ it is necessary to get probability of $p<0.0083$ from ANOVA). Results of paired sample t-test analyses are presented in Tables 14 to 16. For the control group and the 60 dBA group, there was a significant difference for all comparisons made except Stage 1-Stage 2. This indicates that the background sound level of 47.5 dB and the auditory toughening level of 60 dBA did not cause a significant change in ABR thresholds. Conversely, for the 72 dBA group there was a significant difference for Stage 1-Stage 2. There was no significant difference in ABR thresholds when comparing Stage 1-Stage 4 and Stage 2-Stage 4. This indicates a full recovery for the 72 dBA group following the traumatic sound exposure.

Similar analysis was performed to asses the significance of means between experimental groups for each stage separately. The results are presented in Table 17. These results allow for the performance of independent sample t-tests to determine the significance of the difference between the means for specific groups separately within each stage. In this case the Bonferroni Correction was 3 (e.g., to have $p<0.05$ it is necessary to get probability from ANOVA $p<0.017$). There was a significant difference for Stage 1 (baseline) of the 60 dBA group compared to the 72 dBA group ($t(13)=-2.36$, $p<0.05$) and the control group ($t(12)=2.83$, $p<0.05$). This confirms the results of previous

Table 13. ANOVAs with Repetition Performed for Each Experimental Group Separately Assessing the Significance of Stage Variable for ABR

Group	F	df	Significance
Control	94.4	3/3	0.002
60 dBA	272.50	3/5	0.000
72 dBA	254.67	3/4	0.000

Table 14. Results of Paired Sample T-test Comparisons Between Individual Stages for the Control Group for ABR*

Pairs	t	df	Significance
Stage 1-Stage 2	-1.15	5	NS
Stage 1-Stage 3	-14.04	5	0.000
Stage 1-Stage 4	-4.54	5	0.006
Stage 2-Stage 3	-19.36	5	0.000
Stage 2- Stage 4	-5.02	5	0.004
Stage 3-Stage 4	5.44	5	0.003

*significance is presented before the Bonferroni Correction in this figure and all subsequent paired sample comparisons.

Table 15. Results of Paired Sample T-test Comparisons Between Individual Stages for the 60 dBA Group for ABR

Pairs	t	df	Significance
Stage 1-Stage 2	-2.19	7	NS
Stage 1-Stage 3	-21.80	7	0.000
Stage 1-Stage 4	-7.28	7	0.000
Stage 2-Stage 3	-25.86	7	0.000
Stage 2- Stage 4	-7.30	7	0.000
Stage 3-Stage 4	11.61	7	0.000

Table 16. Results of Paired Sample T-test Comparisons Between Individual Stages for the 72 dBA Group for ABR

Pairs	t	df	Significance
Stage 1-Stage 2	-3.32	6	0.016
Stage 1-Stage 3	-13.77	6	0.000
Stage 1-Stage 4	-2.28	6	NS
Stage 2-Stage 3	-18.31	6	0.000
Stage 2- Stage 4	-0.20	6	NS
Stage 3-Stage 4	21.60	6	0.000

Table 17. Results of One-way ANOVA for ABR Thresholds for Different Stages of the Experiment for ABR

Stage	F	df	Significance
Baseline (1)	4.86	2/20	0.02
After toughening or saline injections (2)	11.14	2/20	0.001
After sound exposure (3)	0.44	2/20	NS
After 10 to 12 day recovery (4)	7.91	2/20	0.003

analyses. In addition, a significant difference was found for Stage 2 (after toughening) for the 60 dBA group as compared to the 72 dBA group ($t(13)=4.86$, $p<0.001$) and the control group ($t(12)=2.39$, $p<0.05$). There was a significant difference for Stage 4 (recovery) between the control group and the 72 dBA group ($t(11)=3.80$, $p<0.05$) as well as between the 60 dBA group and the control group ($t(12)=2.33$, $p<0.05$). This suggests that auditory toughening at both 60 dBA and 72 dBA has an effect on ABR thresholds after recovery of a traumatic sound exposure. There was not a significant difference for Stage 4 between the 60 dBA and 72 dBA experimental groups.

Results of the ANOVA with repeated measures for DPOAE amplitudes at all frequencies tested are similar to ABR results, as shown in Table 18, except for the lack of significance for the frequency variable. It should be noted that all data for 15,984 Hz and 14,250 Hz was not analyzed due to DPOAE amplitudes being below the noise floor; therefore, most data points at these frequencies were missing. There was a significant difference in DPOAE measurements for the stage variable ($p<0.000$). The interaction between the three groups and the stages of the experiment at which the measurements were taken was significant ($p<0.05$). This indicates that the DPOAE amplitudes measured during the four stages were different for the three groups. Unlike ABR thresholds, there was no significant difference in DPOAE amplitudes across frequencies and groups. This may be due to the high variability within groups and the fact that the highest frequencies could not be included in the analysis due to missing data.

Similar to ABR results, DPOAE measurements indicated that all interactions with frequency were not significant. Therefore subsequent analyses were performed by collapsing frequencies and using the mean DPOAE amplitude for each animal.

Table 18. ANOVA with Repeated Measures for DPOAE for All Frequencies *

Variable	F	df	Significance
Stage	139.75	3/6	0.000
Frequency	6.24	6/3	NS
Group	93.20	1/8	NS
Stage*Group	3.44	6/14	0.03
Frequency*Group	6.24	12/8	NS

*SPSS did not calculate Stage*Frequency and Frequency*Group*Stage interactions due to missing data points at 15,984 Hz and 14,250 Hz.

The averaged over frequency DPOAE amplitudes are presented in Figure 8. Visual inspection of the results suggested a similar pattern as the ABR results; however, there was not a clear effect of group variable. This is consistent with results of ANOVA presented in Table 19 (no significant group effect and borderline significant Stage*Group interaction).

Analysis of variance with repetition was performed for each group separately (Table 20). The stage variable is significant within all three groups. A set of paired sample t-tests were performed to determine the significance of the difference between the means for specific stages analyzed separately within each group. As with ABR, the Bonferroni Correction method was used. Results of the paired t-test analyses are presented in Tables 21 to 23. For the control group, there was a significant difference for all stages compared except Stage 3-Stage 4. This indicates there was not a significant recovery for the control group. For the 60 dBA group, there was a significant difference between all stages compared. Conversely, there was no significant difference comparing Stage 2-Stage 4 for the 72 dBA group. This indicates that the 72 dBA group recovered to ABR thresholds measured after auditory toughening.

As with ABR, a one-way ANOVA was performed to assess the significance of the means between the experimental groups for each stage separately. These results are shown in Table 24. There was no significant difference between any of the stages between groups. This may be due to the high variability within each group.

Differences in ABR thresholds across all three groups as compared to baseline measurements were also calculated (Figure 9). The 72 dBA, 60 dBA and 47.5 dBA represent the three groups. The control group was exposed to 47.5 dB ambient

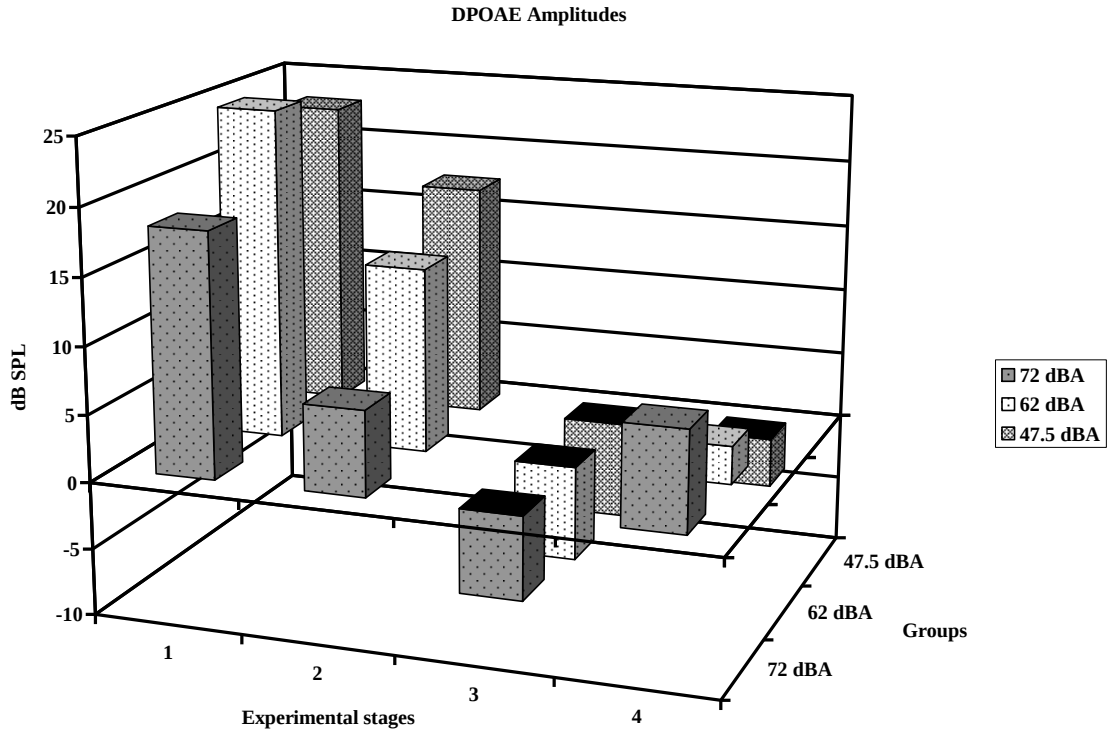

Figure 8. Mean over frequency DPOAE amplitudes for the three groups at each stage in the experiment. The four experimental stages are shown on the x-axis and the three groups are shown on the y-axis. The mean DPOAE amplitudes are measured in dB SPL shown on the z-axis.

Table 19. ANOVA with Repeated Measures for Mean Over Frequency DPOAE Data

Variable	F	df	Significance
Stage	202.74	3/16	0.000
Stage*Group	2.36	6/34	0.052

Table 20. ANOVAs with Repetition Performed for Each Experimental Group Separately Assessing the Significance of Stage Variable for DPOAE

Group	F	df	Significance
Control	65.42	3/3	0.003
60 dBA	173.41	3/5	0.000
72 dBA	22.77	3/4	0.006

Table 21. Results of Paired Sample T-test Comparisons Between Individual Stages for the Control Group for DPOAE

Pairs	T	df	Significance
Stage 1-Stage 2	3.17	5	0.025
Stage 1-Stage 3	17.27	5	0.000
Stage 1-Stage 4	9.28	5	0.000
Stage 2-Stage 3	11.08	5	0.000
Stage 2- Stage 4	7.38	5	0.001
Stage 3-Stage 4	-1.85	5	NS

Table 22. Results of Paired Sample T-test Comparisons Between Individual Stages for the 60 dBA Group for DPOAE

Pairs	t	df	Significance
Stage 1-Stage 2	2.66	7	0.033
Stage 1-Stage 3	24.88	7	0.000
Stage 1-Stage 4	7.78	7	0.000
Stage 2-Stage 3	5.45	7	0.001
Stage 2- Stage 4	2.32	7	0.053
Stage 3-Stage 4	-3.25	7	0.014

Table 23. Results of Paired Sample T-test Comparisons Between Individual Stages for the 72 dBA Group for DPOAE

Pairs	T	df	Significance
Stage 1-Stage 2	2.95	6	0.026
Stage 1-Stage 3	9.75	6	0.000
Stage 1-Stage 4	2.67	6	0.037
Stage 2-Stage 3	3.02	6	0.024
Stage 2- Stage 4	-0.09	6	NS
Stage 3-Stage 4	-3.30	6	0.016

Table 24. Results of One-way ANOVA for DPOAE Amplitudes for Different Stages of the Experiment

Stage	F	df	Significance
Baseline (1)	2.43	2/20	NS
After toughening or saline injections (2)	1.92	2/20	NS
After sound exposure (3)	1.21	2/20	NS
After 10 to 12 day recovery (4)	2.50	2/20	NS

background noise. Each vertical bar represents the difference in intensity for three Stages 2 through 4. Figure 9 illustrates in a different manner the results of previous ABR analyses.

The correlation of the extent of ABR threshold shift from auditory toughening and after ABR recovery measured 10 to 12 days after the traumatic sound exposure with the background sound level is shown in Figures 10 and 11 respectively. These intensity levels are sound levels measured as dBA for the three different groups. The y-axis shows the amount of change after toughening for each group (Figure 10) or the extent of improvement 10 to 12 days after the traumatic sound exposure (Figure 11). The linear regression line shows that as intensity increases the threshold shift after auditory toughening increases (Figure 10) and the amount of protection that is exhibited also increases (Figure 11). Calculations were performed using the average thresholds of each animal. However, for clarity of the presentation only the mean calculations of all animals were plotted in the figures. There is a statistically significant correlation for both ABR threshold shifts after auditory toughening with background sound level ($r=0.453$, $t(19)=2.22$, $p<0.05$) and the extent of ABR recovery ($r=0.716$, $t(19)=4.47$, $p<0.001$). These correlations suggest that a) the more intense the auditory toughening signal the greater the threshold shift after toughening and b) the 47.5 dBA ambient background noise may still be offering protection from a traumatic sound exposure as there is a proportional decrease of a protective effect while the sound level decreased from 60 dBA to 47.5 dBA.

Differences in DPOAE amplitudes across all three groups as compared to baseline measurements were also calculated (Figure 12). As with ABR, all three groups had

similar threshold shifts following the traumatic sound exposure; however, the 72 dBA group recovered more than the 60 dBA and the control groups.

The correlation of the extent of DPOAE shift following auditory toughening and after DPOAE recovery measured 10 to 12 days after the traumatic sound exposure with the background sound level are shown in Figures 13 and Figure 14 respectively. As with ABR correleations, calculations were performed using the average thresholds of each animal. Results are similar to ABR correlations, as intensity increases the threshold shift after auditory toughening increases (Figure 13) and the amount of protection that is exhibited also increases (Figure 14). However, the correlation was not significant for DPOAE shifts after auditory toughening with background sound level ($r=0.257$, $t(19)=1.16$, NS). The correlation for the extent of DPOAE recovery was of borderline significance ($r=0.424$, $t(19)=2.04$, $p=0.055$). There correlations are not as strong as ABR correlations which may be due to the high variability within subjects and the omission of the highest frequencies.

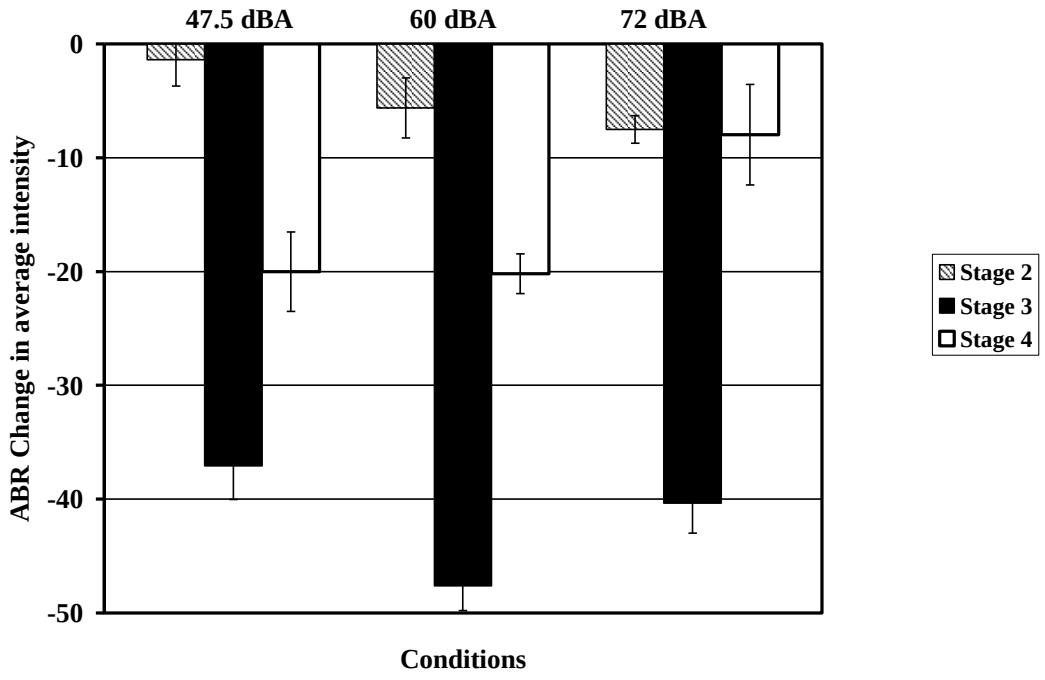

Figure 9. Differences in ABR thresholds compared to baseline measurements. For each animal the difference between the threshold for a given stage and baseline (Stage 1) was evaluated and then the average for a group of animals was calculated. The three groups are on the x-axis. The change in average intensity from the baseline is on the y-axis. Each vertical bar represents the experimental stages (after toughening, after traumatic sound, and 10 to 12 day recovery) in which the rats were tested.

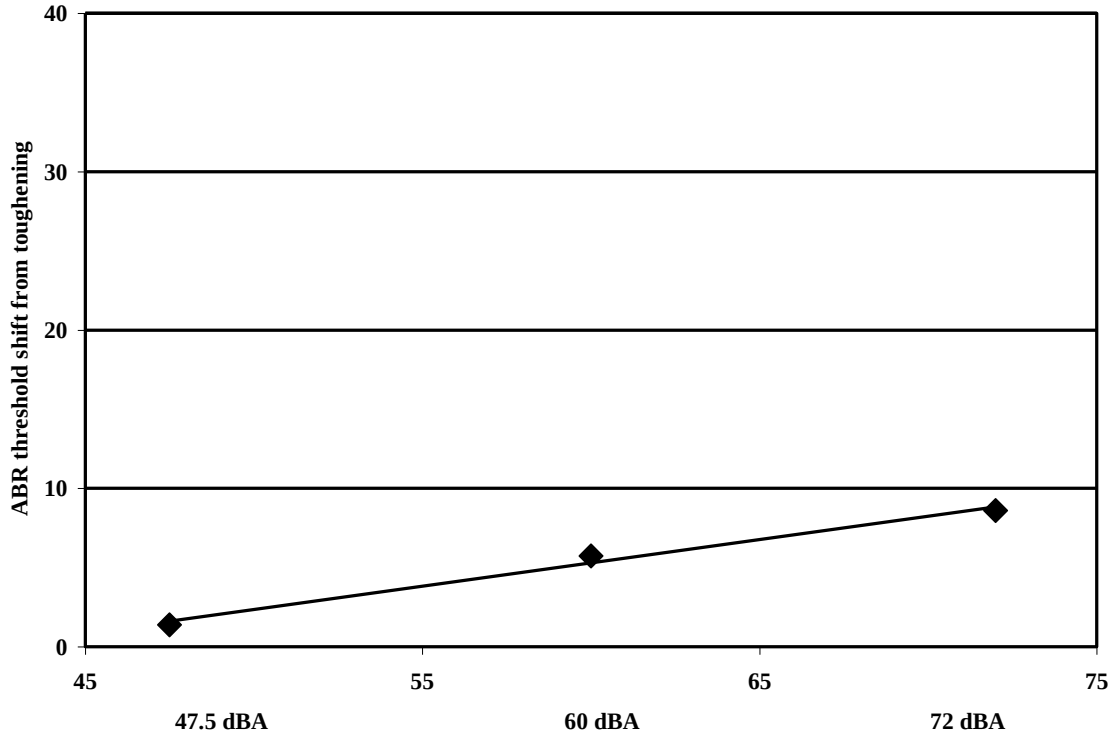

Figure 10. Correlation of the extent of ABR threshold shift following auditory toughening with background sound level. The x-axis represents the intensity levels of the three groups and the y-axis represents the difference in mean ABR thresholds after toughening or background sound level. The correlation is statistically significant ($r=0.453$, $t(19)=2.22$, $p<0.05$). Calculations were made using the individual data from all subjects; however, for clarity of the presentation this figure and all subsequent correlations show the mean data for each group.

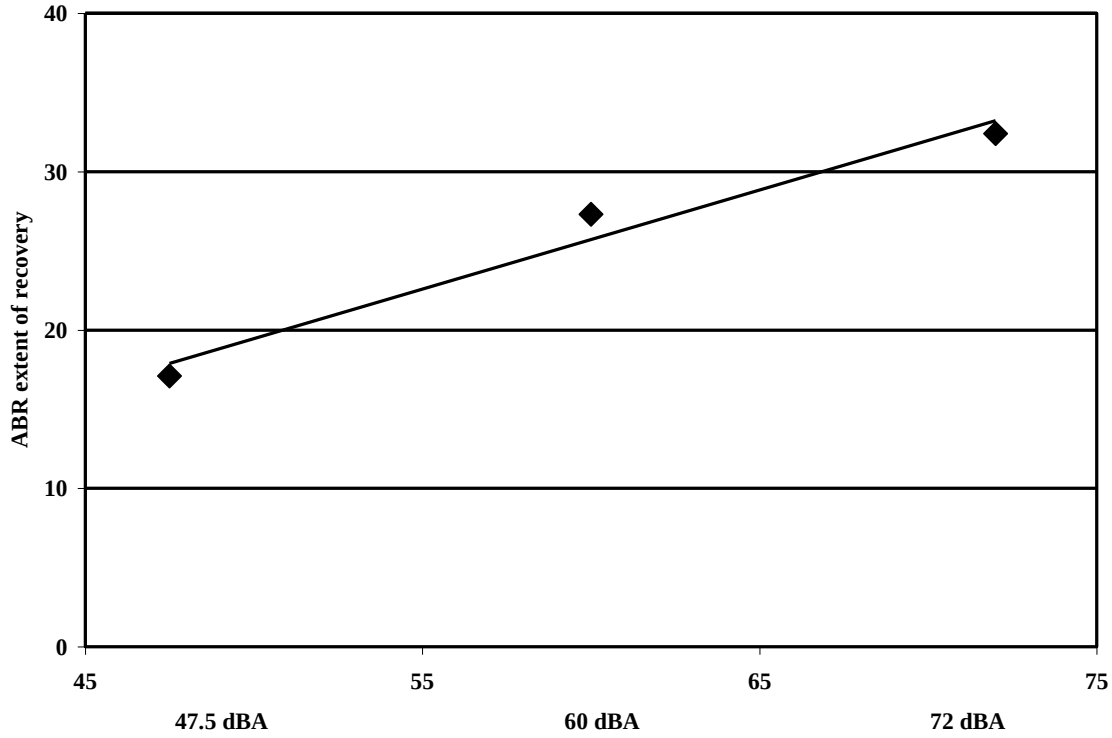

Figure 11. Correlation of the extent of ABR recovery 10 to 12 days after sound exposure with background sound level. The x-axis represents the intensity levels of the three groups and the y-axis represents the amount of recovery measured 10 to 12 days after the traumatic exposure. The correlation is statistically significant ($r=0.716$, $t(19)=4.47$, $p<0.001$).

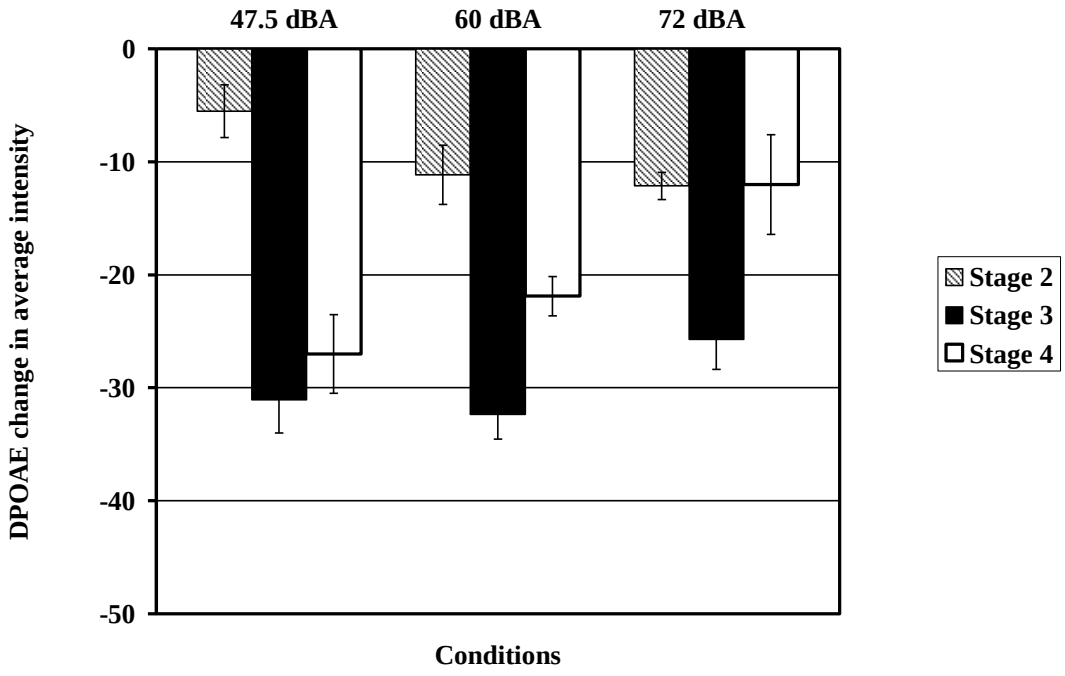

Figure 12. Differences in DPOAE responses compared to baseline measurements. DPOAE differences in amplitudes were calculated in an identical manner as for ABR. The three groups are on the x-axis. The change in average intensity from the baseline is on the y-axis. Each vertical bar represents the experimental stages (after toughening, after traumatic sound, and 10 to 12 day recovery) in which the rats were tested.

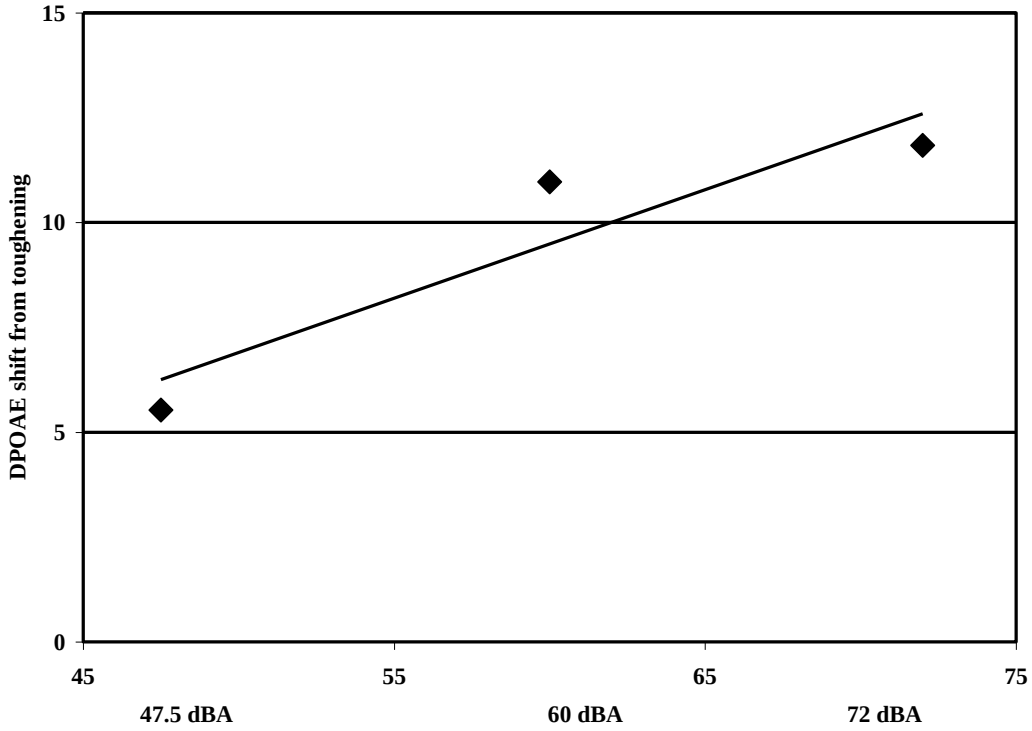

Figure 13. Correlation of the extent of DPOAE shift following auditory toughening with background sound level. The x-axis represents the intensity levels of the three groups and the y-axis represents the difference in mean DPOAE amplitudes after toughening or saline injections compared to baseline measurements. The correlation was not statistically significant ($r=0.257$, $t(19)=1.16$, NS)

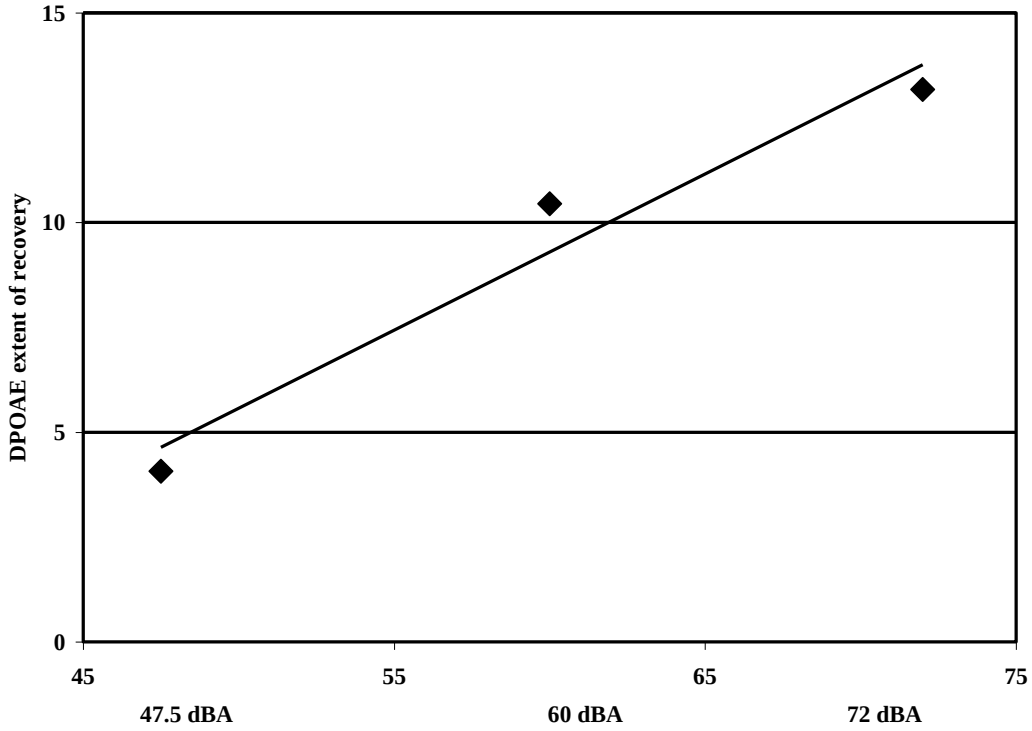

Figure 14. Correlation of DPOAE recovery 10 to 12 days after sound exposure with background sound level. The x-axis represents the background sound intensity levels of the three groups and the y-axis represents the amount of recovery measured 10 to 12 days after the traumatic exposure. The correlation is of borderline significance ($r=0.424$, $t(19)=2.04$, $p=0.055$).

CHAPTER 5

DISCUSSION

Overview

The results of this study have shown that protection against a traumatic sound can occur by exposing rats to a low level BBN of either 60 dBA or 72 dBA 12 hours per day, for 5 days. The protection is manifested by a larger reduction in ABR thresholds and DPOAE amplitudes of the rats in the experimental groups 10 to 12 days after the traumatic sound exposure (7.8 kHz pure tone at 110 dB SPL for 20 minutes) as compared to measurements of the rats that were not conditioned. Animals exposed to a toughening level of 72 dBA exhibited a greater reduction in threshold shifts than animals exposed to a 60 dBA toughening stimuli 10 to 12 days after the traumatic sound exposure. Interestingly, there was no significant difference in shifts for ABR thresholds and DPOAE amplitudes immediately following the traumatic sound exposure for all three groups. This indicates that toughening influences mechanisms responsible for chronic recovery but not mechanisms involved in short term, acute effects.

Presented results differ from literature in showing protective effects of a much lower level of toughening sound, i.e. 60 dBA vs. previously reported effects seen only for sounds above 85 dB SPL. This discrepancy may have resulted for several reasons. The present study was conducted on rats, whereas all other studies reviewed used chinchillas. Another confounding factor is that the results from these studies are variable and the use of different parameters makes the results difficult to interpret and compare.

Auditory Toughening Parameters

Auditory toughening levels as low as 60 dBA appear to still yield protection from a traumatic sound in rats. Note, that the highest toughening intensity used, 72 dBA, was still lower than the lowest intensity levels used in other sound conditioning or auditory toughening studies. The research which has been conducted on chinchillas to determine the effect of toughening used significantly higher intensity levels ranging from 85 to 95 dB SPL (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1992; Subramaniam et al., 1993b). On the other hand, sound conditioning, the use of continuous stimuli, uses lower intensity levels ranging from 78 to 89 dB SPL to condition mice and guinea pigs to a traumatic exposure (Canlon et al., 1988; Canlon & Fransson, 1998; Yoshida & Liberman, 2000). Note, however, that these levels were still above the highest level used in this study. It is not clear if a protective effect will be observed in chinchillas using the same sound level as were used in this study. The results of the present study launch a possibility that low level background sound may offer significant protection against subsequent traumatic sound exposures. It is still not clear if there is a minimum threshold level for the protective effect of sound and future studies are needed to clarify this issue.

In the present study, a high frequency BBN toughening stimulus produced a toughening effect at all frequencies. Similarly, a low frequency toughening stimulus (500 Hz OBN) produced similar effects across all frequencies (Campo et al., 1991). Both high and low frequency toughening stimuli have provided protection from a subsequent traumatic sound.

In the present study, toughening for 12 hours per day for only 5 days yielded protection against a traumatic exposure. The majority of the literature has used toughening durations of at least 10 days for 6 hours per day (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1992; Subramaniam et al., 1993b). The results of the present study indicate that toughening for a shorter period of time for a longer time interval is still effective. It is not clear as to what the minimal duration of sound exposure per day is, that would still offer a protective effect.

Traumatic Exposure Parameters

In the literature the same stimulus has been used for toughening and traumatic sound with significantly higher intensity levels for the traumatic noise. However, for the present study two different types of stimuli were used as it better reflects real life situations. The toughening stimulus was BBN, whereas the traumatic sound exposure was a pure tone. The highest intensity of the BBN was either 60 or 72 dBA (61.15 or 73.15 dB SPL) for an octave band centered at 8 kHz and the traumatic exposure was a 7.8 kHz pure tone presented at 110 dB SPL.

The results of the present study suggest that auditory toughening can yield protection against a high intensity sound lasting 20 minutes. Other studies have used a traumatic sound lasting up to 48 hours. Higher traumatic exposure intensities for longer durations of time have been used on chinchillas (Campo et al., 1991; McFadden et al., 1997; Subramaniam et al., 1992; Subramaniam et al., 1993b). It is possible to speculate that the extent of threshold shift and outer hair cell function should be used to compare the results of various experiments rather than sound intensity and duration of the toughening and traumatic sound stimuli. Various species exhibit different resistance to

sound. The use of psychoacoustical descriptions of damaging sound might be not be an optimal strategy. Notably, the damage induced by the traumatic sound exposure in the present study seems to be similar to that presented in other studies.

Minimal research regarding auditory toughening has been conducted in rats. Chinchillas have been the primary animals used to study the effects of auditory toughening. At the same time, rats are recognized as excellent subjects for behavioral and electrophysiological studies of the auditory system. The use of chinchillas in previous studies results from a variety of factors, including easy access to the round window of the cochlea, a factor which was irrelevant for the present study.

As with other studies, the greatest threshold shift occurred immediately after the traumatic sound exposure. Interestingly, auditory toughening at 60dBA or 72 dBA does not have a protective effect on hearing threshold immediately following a traumatic sound exposure, suggesting protective mechanisms involve long term survival and recovery of hair cells rather than immediate protection.

In the present study a protective effect was seen at all frequencies for both 60 dBA and 72 dBA toughening intensities. Conversely, Campo et al. (1991) only found a protective effect between 500 Hz and 4 kHz when the toughening and traumatic exposures were a 500 Hz OBN stimulus.

Subramaniam et al. (1992) found that a protective effect was no longer seen after a rest period of 5 days between auditory toughening and the traumatic exposure. However, a rest period of 18 hours yielded protection against a traumatic exposure. Conversely, a protective effect was measured using a 5 day rest period by Campo et al. (1991). A 5 day rest period was chosen to make sure that hearing thresholds had

recovered to baseline thresholds after the toughening stimuli. Following a 5 day recovery period from toughening, thresholds had returned to normal (McFadden et al., 1997).

Results of the present study reveal that even after a 48 hour rest period protection from a traumatic sound is still seen in rats. Interestingly, a rest period of 48 hours following sound conditioning did not reveal significant changes in threshold shifts between the control and experimental groups in mice (Yoshida and Liberman, 2000). Further studies are needed to determine the amount of time after conditioning or toughening in which the protective effect is no longer observed.

Future Research

There has been a substantial amount of research completed in the area of auditory toughening; however, the use of various stimulus parameters have made it difficult to compare studies. Further research should be completed regarding the minimum toughening intensity needed to exhibit a protective effect from a traumatic sound exposure to determine if protection occurs at toughening levels below 60 dBA. The results of the present study suggest that exposure to any sound may provide some protection from a traumatic exposure. Future research should include exposing animals to extremely low levels of sound (i.e. an auditory booth) and to gradually increase sound levels to determine if merely avoiding excessive silence will provide protection from a traumatic exposure.

Further studies are needed to determine the intensity of the traumatic sound for which a given auditory toughening intensity still provides protection. In the present study, a 110 dB SPL traumatic sound exposure was used. Futures studies should include

higher and lower traumatic sound intensities using the same toughening stimuli to determine and compare the extent of protection.

Tinnitus is often induced by noise exposure and accompanied by hearing loss. If a protective effect regarding hearing loss is observed following auditory toughening, is it possible that the same effect could be observed for tinnitus? To date, there is no research regarding auditory toughening and its effects on tinnitus. Future studies regarding tinnitus and toughening are needed.

Norena and Eggermont (2005) studied the effects of low level sound after exposure to a traumatic sound in cats. Hearing loss for cats exposed to an enriched level of sound after a traumatic sound occurred in a smaller frequency range (6 kHz through 8 kHz) compared to animals not exposed to an enriched level of sound (6 kHz through 32 kHz). Norena and Eggermont found that protection can still occur after exposure to a traumatic sound. Future studies should be conducted to compare the recovery when auditory toughening occurs before the traumatic sound exposure to the results of toughening after the traumatic sound exposure. This would be similar to a variety of sound therapies used in tinnitus treatment.

Application

The knowledge that auditory toughening could potentially protect the cochlea from subsequent exposures may have a significant effect on preventing NIHL. Exposure to a low level stimulus prior to a traumatic exposure has shown to decrease the amount of hearing loss incurred by that traumatic exposure in animals. In the future, the use of auditory toughening may be applied to humans and could potentially be another form of commonly used oto-protection. Auditory toughening would be especially useful to

individuals who cannot use hearing protection devices or individuals who need additional hearing protection.

APPENDIX

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