The Evaluation of Frequency Specific Cochlear Damage:

Measurement of Low Frequency Hearing Function

by

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Abstract

Background

Sensorineural hearing loss is a highly prevalent condition. A common cause of this type of loss is noise exposure (noise-induced hearing loss; NIHL). Effective management is available for NIHL, however, appropriate management depends on accurate diagnosis. Three tests commonly used to make a clinical diagnosis of hearing loss are distortion product otoacoustic emissions (DPOAE), auditory brainstem responses (ABR), and the cochlear microphonic (CM). In general, these tests are more sensitive to hearing loss at higher frequencies than at lower frequencies. The evaluation of lower frequency hearing function is as important as that of higher frequency hearing function; however, the use of these tests in diagnosing low frequency hearing loss has not been adequately addressed in the research literature. To address this gap in knowledge, the current study was conducted.

Research Questions

1. What are the response patterns of the auditory system tests in the presence of normal hearing (Group 1) and NIHL?
2. What are the differences in the three tests as a function of NIHL, specifically the following:
    a. low frequency band-noise exposure (Group 2)
    b. high frequency band-noise exposure (Group 3)
    c. a sequence of low then high frequency band-noise exposure (Group 4), and,
    d. a sequence of high then low frequency band-noise exposure (Group 5)?
3. What is the relationship between the CM measured at the round window (RW) and the CM measured at the ear canal (EC) in the presence of normal hearing and NIHL?

4. What are the differences in the morphology and number of outer hair cells (OHCs) as a result of NIHL?

Methods

Fifteen guinea pigs were equally allocated into five groups (i.e., normal control and four kinds of noise exposure). DPOAE, ABR, and CM (at RW and EC) were used to evaluate hearing function in the five groups. Each test was measured at 0.5 kHz, 2 kHz, 4 kHz, 6 kHz and 8 kHz. After the tests were completed, the animals were euthanized, the cochlea were removed for silver nitrate staining, and the hair cells were examined under microscope.

Results

Research questions 1 and 2. Response patterns of the auditory system tests were compared in the presence of normal hearing and NIHL. The highest signal-to-noise ratio (SNR) of DPOAE and the highest amplitudes of ABR and CM occurred in the control group. At 0.5 kHz and 2 kHz, there were significant reductions in SNR of DPOAE and in the amplitudes of ABR and CM in groups 2, 4, and 5. At 6 kHz and 8 kHz, there were significant reductions in the SNR of DPOAE and the amplitudes of ABR and CM in groups 3, 4, and 5.

There was no significant difference in hearing loss between single and double frequency band noise exposure. At 0.5 and 2 kHz, there were no significant difference between groups 2, 4, and 5; at 6 and 8 kHz, there was no significant difference between groups 3, 4, and 5.

Research question 3. There was a statistically significant association between CM RW and CM EC in all test groups at all frequencies.

Research question 4. There were no significant differences in the total number of missing OHCs between the five groups. Slight morphological changes were observed in the noise exposure groups.

Discussion

The evaluation of low frequency hearing function, particularly the successful recording of 0.5 kHz DPOAE and CM, is an important contribution to the literature on evaluation of low frequency hearing function. The significant correlation between the CM EC and CM RW indicates that the CM EC preserved the essential characteristics of CM RW, providing support for EC recording in clinical applications. The lack of a statistically significant difference between one and two frequency band-noise-exposure may be because relatively high levels of noise intensity and longer durations of exposure than were used in the current study are required to produce significant effects on hearing function. Finally, the lack of a statistically significant difference in the total number of missing OHCs as a function of noise may be explained by the time, i.e., not enough time had passed after the experiment and before the animals were euthanized to see significant changes in numbers of hair cells.

Preface

This dissertation is an original work by YongQiang Yu. The research project, of which this dissertation is a part, received research ethics approval from the University of Alberta Health Research Ethics Board, Health Panel. The title of the research project that received ethics approval was LOW FREQUENCY COCHLEAR ELECTRICAL RESPONSES，No. AUP00000429. This research was supported by operating and infrastructure grants to PI Dr. Ming Zhang.

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I would like to thank my supervisor Dr. Tammy Hopper, my committee members Dr. Ming Zhang and Dr. Bill Hodgetts, thanks for your mentoring me in the processes of doing research; I would like to acknowledge all people and institutions that have helped me complete my doctorate study, I wish to express my sincere appreciation for your indispensable support, the greatest appreciation is from the bottom of my heart.

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List of Abbreviations

ABR = auditory brainstem responses

CM = cochlear microphonic

DPOAE = distortion product otoacoustic emission

RW = round window

EC = ear canal

SNR = signal noise ratio

SNHL = sensorineural hearing loss

SPL = sound pressure level

NIHL = noise induced hearing loss

Group 1 = control group without noise exposure

Group 2 = group with low frequency band-noises exposure

Group 3 = group with high frequency band-noises exposure

Group 4 = group with low then high frequency band-noises exposure

Group 5 = group with high then low frequency band-noises exposure

Chapter 1: Introduction

Proper diagnosis of location of hair cell damage in the cochlea, addressed in this thesis, is important in the clinical setting, as such damage is the most significant cause of hearing loss in all ages (Mills, 1982). Such damage can be caused by many factors, including noise. About 500 million people around the world are at risk of developing noise induced hearing loss (NIHL), which may compromise health, employment opportunities and quality of life (Alberti, 1998; Henderson, 2011). NIHL is one kind of sensorineural hearing loss (SNHL). Treatments are available in several forms, including hearing aids and/or cochlear implants; however, a necessary first step in management is accurate diagnosis. Pure tone audiometry can provide hearing thresholds according to the responses of the patient to acoustic stimuli, and is a common way to evaluate hearing function. However, because pure-tone audiometry relies upon the subjective judgement of the patient, it is not always accurate (Tyler, 1980); also, other more objective auditory system tests may aid in the diagnosis of hearing loss.

Both high and low frequency SNHL must be evaluated. However, objective tests of the auditory pathway such as electrocochleography (ECoG), ABR, and otoacoustic emission (OAE) may not be accurate at detecting sounds at low frequencies, particularly when the measured frequency is below 1 kHz (Gorga, 1993; Picton, 2007; Sininger, 2007; Spoor, 1976). Thus, the primary purpose of this study was to determine the accuracy of these objective tests for detecting low frequency SNHL. The second purpose was to establish the response patterns of the auditory system tests to provide a direct illustration of the overall auditory system function across all the measured frequencies, and the third purpose was to compare specific ECoG recordings (i.e., the cochlear microphonic) at different locations in the ear.

Different types of NIHL were also of interest. In daily living, people may be exposed to low frequency noises or high frequency noises, or exposed to mixed noises in which low and high frequency noises are combined. However, it is unknown whether two-frequency NIHL is worse than the one-frequency NIHL (Charles, 1973; Mills, 1978; Murnane, 2003; Zhang, 1997, 2010, 2012a). Thus, addressing this issue was the fourth purpose of this study. Examination of the effects of exposure to noise on the hair cells of the inner ear was the fifth purpose of this thesis.

In the following sections, a broad overview is provided of ear anatomy and physiology, hearing function and the auditory system tests used in this study. A review of research related to the thesis concludes the section.

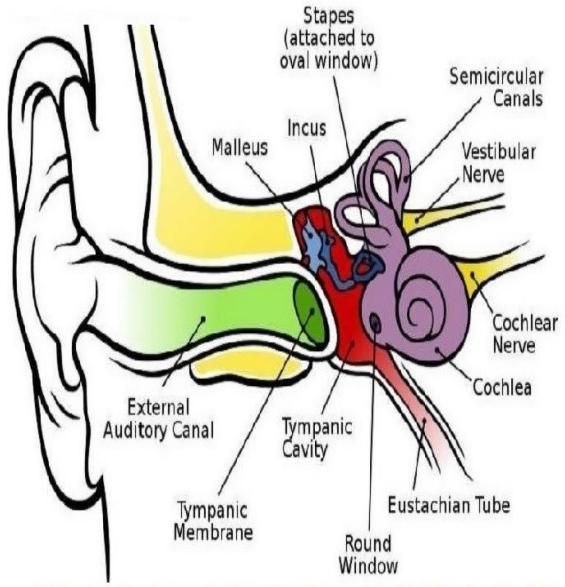
1.1. Ear Anatomy

The mammalian ear has three parts: outer, middle and inner ear, which are illustrated in Figure 1-1. The outer ear consists of the pinna and external ear canal. The middle ear includes the tympanic membrane and ossicular chain. The tympanic membrane is situated between the outer and middle ear. The ossicular chain connects the tympanic membrane to the inner ear, and the chain is composed of three tiny ossicles, called malleus, incus and stapes. The malleus is attached to the tympanic membrane; the footplate of the stapes is attached to the round window membrane, a flexible membrane which covers the oval window of the cochlea.

The inner ear contains the vestibule (for balance) and cochlea (for hearing). The cochlea, a snail-shaped structure, is divided into three chambers by the basilar membrane, the scala vestibule, scala tympani, and scala media. Each chamber is filled with lymph fluid. In the scala media the fluid is endolymph, whereas in the other two chambers the fluid is perilymph.

In the scala media, highly sensitive hair-like cells (‘hair cells’) are rooted in the basilar membrane. Each hair cell has rod-like structures on its top surface, made of protein called

stereocilia. Stereocilia emerge from the top surface of hair cells, and are immersed in endolymph. The hair cells can be further divided into two groups, inner hair cells (IHCs) and outer hair cells (OHCs). The bases of IHCs form synapses with spiral ganglion neurons, whose axons constitute the auditory nerve fiber. Another important structure is the tectorial membrane, a gel-like structure overlaid on the hair cells (Figure 1-2) (Freeman, 2003, a and b; Ghaffari, 2007; Goodyear, 2002; Gueta, 2006; Richter, 2007).



Chittka, L. & Brockmann, A. Perception Space – The Final Frontier, PLoS Biology. 2005, 3(4): e137. Open-access article (Creative Commons Attribution License).

Figure 1-1. Anatomy of the Human Ear

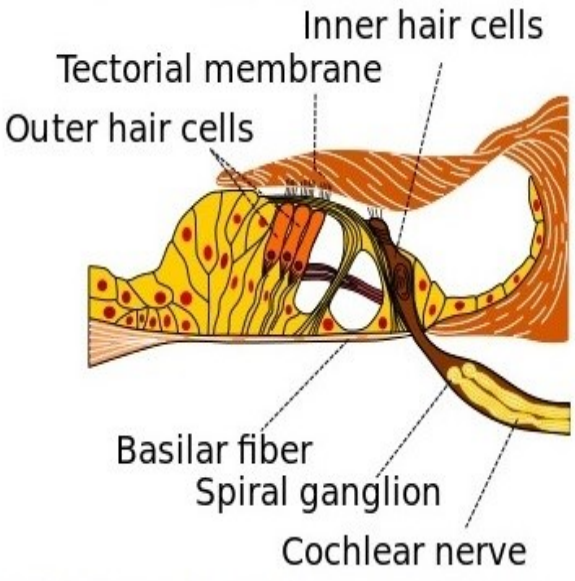


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Figure 1-2. The Tectorial Membrane

The basilar membrane is the second gel-like structure of the inner ear and has graded mechanical properties. At the cochlea base, the basilar membrane is narrow and stiff, and sensitive to high frequency acoustic stimuli. At the cochlear apex, the membrane is wide and flexible, and sensitive to low frequency acoustic stimuli (Robles, 2001; Shera, 2007). The graded properties are illustrated in Figures 1-3 and 1-4.

The basilar membrane and hair cells form a complex tunnel-like structure, through which cochlear nerves travel and carry the auditory signals to the brain. This complex structure is the Organ of Corti (Nuttall, 1995).

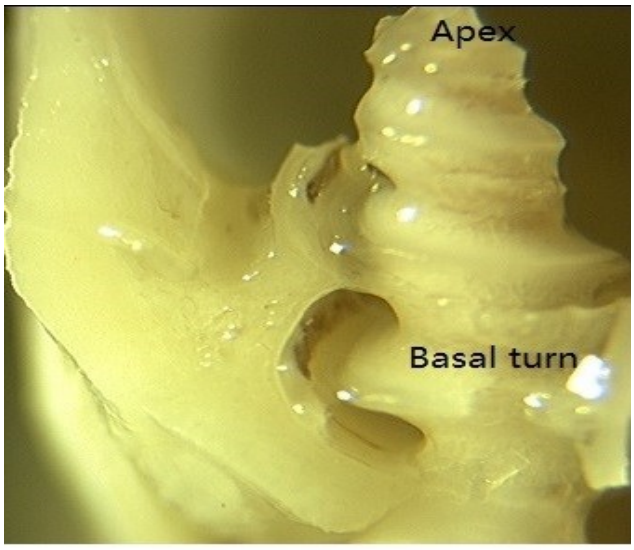


Figure 1-3. The Guinea Pig Cochlea

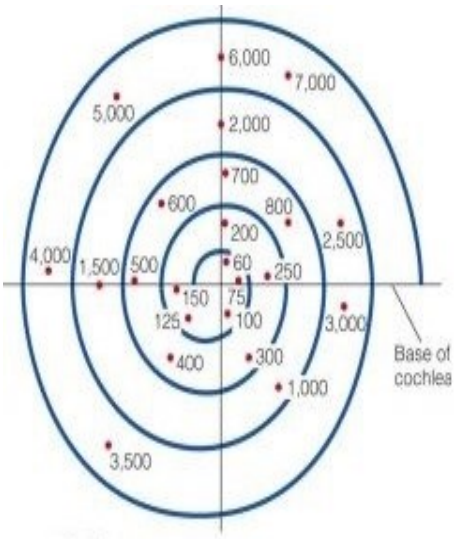


Figure 1-4. The Tonotopic Map of the Guinea Pig Cochlea

1.2. Physiology of Normal Hearing

In the process of normal hearing, sound waves travel through the ear canal and cause vibration of the tympanic membrane. The ossicular chain then transmits the vibration to the round window membrane, causing the round window membrane to make a piston-like movement. This inward and outward movement of the round window membrane induces pressure oscillations in the cochlear lymph fluid, initiating traveling waves of displacement along the basilar membrane (Gan, 2007).

The traveling wave of the basilar membrane moves the hair cells toward or away from the tectorial membrane. At the same time, the oscillation of lymph fluid causes the stereocilia to deflect

as hair cell bases are rooted in the basilar membrane. Such deflection of the stereocilia in one direction increases the release of neural transmitters at the bases of IHCs, while the deflection in the other direction inhibits the release. At this stage, the acoustic energy of the sound wave is transformed into the chemical energy of the neural transmitters (Karavitaki, 2010).

The accumulation of the neural transmitter at the bases of the IHCs increases the neuro-electrical activity of the spiral ganglion neurons, whereas the reduction of the transmitter inhibits this activity. At this stage, the chemical energy of the neural transmitter changes into the neuro-electrical currents of the auditory nerve (Appler, 2011).

In the hearing process, IHCs are sensory receptors that are responsible for receiving and processing external sound, and transmitting 95% of auditory signals to the primary auditory cortices of the brain. OHCs expand and contract in response to external acoustic stimuli, working like motor units. These mechanical movements of the OHCs enhance the traveling waves of displacement along the basilar membrane, increase the deflection of the stereocilia of IHCs, and eventually amplify the neuro-electrical currents transmitted to the brain. The OHCs act like amplifiers in the process of auditory transmitting by the IHCs (Anthony, 2012).

The ability of OHCs to expand and contract is very important for their function. The structural basis of this ability is the actin and myosin contained in the OHCs (Rogers, 2011). The OHCs are tuned structures, which allows the cells to expand and contract rhythmically in response to acoustic stimuli. There is only one row of IHCs and three rows of OHCs; it has been estimated that without OHCs, about 40 to 60 decibels of SNHL will occur (Rogers, 2011).

Dysfunction can occur at one or more stages of the hearing process, and can be a result of anatomical or physiological abnormalities. Type or category of hearing loss depends on what stage of the process is disrupted.

1.3. Categories of Hearing Loss

There are three kinds of hearing loss: conductive, sensorineural, and mixed. Mixed hearing loss is a combination of conductive and sensorineural loss (Mills, 2006). Any condition in the outer and/or middle ear that prevents the ear from conducting sound properly is a conductive hearing loss. Conductive loss may be a result of blockage in the ear canal (impacted cerumen), or a middle ear infection (otitis media), for example. Conductive hearing loss is usually mild or moderate in nature (Herrgard, 1995; Mills, 2006).

SNHL can be subdivided into sensory hearing loss and neural hearing loss. Sensory hearing loss results when hair cells are missing or damaged. Neural hearing loss results from damage to the spiral ganglion neurons and/or the auditory nerves. SNHL is usually severe and permanent, and can worsen over the time (Lin, 2012; Mills, 2006). Clinically, sensory and neural hearing losses are collectively called SNHL because the damage of hair cells is usually accompanied by damage to spiral ganglion neurons. The most common causes of SNHL are genetic predisposition, ototoxicity, presbycusis and noise exposure (Sogebi, 2013).

1.4. Noise-induced Hearing Loss

Long-term exposure to intense noises can damage hair cells, and cause permanent hearing loss. The level of intense noise is usually above 75 dB SPL; noises of less than 75 dB SPL are unlikely to damage hearing function even after long-term exposure (Noise-Induced Hearing Loss, NIH Publication No. 14-4233). OHCs are particularly susceptible to noise induced damage (Hu,

2000). Yet, the types of noise presented and the differential effects of low versus high frequency noise exposure have not been fully investigated.

The actual mechanism of how noise causes hearing loss is unclear, although several theories exist (Hunter, 1972, 1973; Keilmann, 2013; Mills, 2006; Nottet, 2009; Spoendlin, 1971). Noise vibration may cause the mechanical destruction of the hair cells and basilar membranes (Clifford, 2009; Hamernik, 1974 a, b; Hawkins, 1976; Hunter, 1972; 1973; 1974; Mulroy, 1998; Spoendlin, 1971). Noise stimulation may increase the formation of mitochondrial free radicals in the cochlea (Lim, 1971; McFadden, 2001; Ohlemiller, 1999a, 1999b, 2000; Yamane, 1995), resulting in damage to DNA and the death of hair cells (Halliwell, 1998). Noise exposure could also reduce the blood supply to the inner ear (Axelsson, 1981, 1987; Duvall, 1987; Haupt, 2002; Hawkins, 1971, 1972; Lipscomb, 1973; Miller, 1996; Perlman, 1962; Scheibe, 1993). If the formation of free radicals and the reductions in blood supply exist simultaneously, there might be synergistic effects between them, causing neural swelling, necrosis and apoptotic death of the hair cells (Henderson, 2006).

Pawelczyk (2009) and Van (2006) contend that genetic predisposition is an important contributing factor to the development of NIHL. At present, the identified genes involved in the development of NIHL regulate the functions of potassium ion channels (Pawelczyk, 2009; Van, 2006) and heat shock proteins (Konings, 2009; Yang, 2006).

In the above theories, genetic susceptibility is a predisposing factor and noise exposure is the pathologic factor in the development of NIHL. Because genetic background is not changeable, attention should be paid to noise prevention and early diagnosis. In the following section, common diagnostic technologies are introduced.

1.5. The Evaluation of Hearing and Auditory System Function

The clinical evaluation of hearing and auditory system function consists of subjective and objective tests. Pure tone audiometry (PTA) is subjective, and although it can provide information on general integrity of the auditory pathway, its subjective nature limits its clinical application. For example, PTA cannot be used with individuals who cannot reliably respond during testing (e.g, neonates, individuals with severe cognitive impairment). Therefore, objective tests are often used, specifically, auditory brainstem response (ABR), otoacoustic emissions (OAEs) and ECoG (i.e., cochlear microphonic; CM).

1.5.1. Auditory Brainstem Response

Auditory evoked neural-electrical responses are commonly used to evaluate auditory neural function. ABR is used to evaluate the general integrity of the auditory system, from the cochlea through the brainstem, and up to the thalamus. The evoked ABR usually has five peaks, P1–P5, and responses can be quantified by calculating the peak amplitudes.

Each of five peaks is composed of the evoked potentials generated from multiple nuclei and neural tracts on the auditory pathway, with each contributing to potentials that form different peaks (Legatt, 1988). Thus, each peak may contain more than one generator's potentials. Because of this lack of specificity in etiology of the potential, ABR can only estimate the general integrity of the auditory system (Legatt, 1988; Moller, 1988). ABR, then, cannot replace hearing tests, and should be used in conjunction with other auditory system measures (Korczak, 2012).

Another auditory evoked potential is the auditory steady state response (ASSR). ASSR is middle latency response, it is more affected by cognitive function; the amplitude of ASSR changes with the arousal state of the subject (Plourde, 1991). It has been reported that sleep reduced the amplitude to between one third and one half of the amplitude during wakefulness; and the

amplitudes would be further reduced by general anesthesia (Plourde, 1991). ASSR is commonly used in humans, however, ASSR was not appropriate for use in the current study as the animals were under general anesthesia during testing.

1.5.2. Electrocochleography

In the cochlea there are two stimulus-evoked receptor potentials, the cochlear microphonic (CM) and summating potential (SP). In addition, other receptor potentials of hair cells include the resting potential (RP), and the compound action potential (CAP) - the potential of the cochlear auditory nerve. CM, SP and CAP are collectively referred to as ECoG (Howard, 1988; Hudspeth, 1982).

The rationale for the clinical usage of ECoG is that when there are pathological changes in the inner ear (e.g., formation of endolymph hydrops), the evoked potentials would be abnormal (Santarelli, 2002; Yokoyama, 1999). However, there are some concerns about this rationale. For example, Meniere's disease was thought to be caused by endolymph hydrops, but not all patients with Meniere's disease had abnormal ECoG. Conversely, some people had abnormal ECoG, but no evidence of endolymph hydrops (Nam, 2004). Thus, the use of ECoG has been found to be neither sensitive nor specific for dysfunction of the inner ear (Honrubia, 1999; Rauch, 1989), and, like ABR, ECoG should be used with other tests for a comprehensive diagnosis of hearing loss and auditory system function.

Although there are four components that comprise ECoG, the CM was adopted in the current research. CAP was not adopted in the current study because ABR focuses on the general function of auditory neural system, including the potential of the cochlear auditory nerve; thus CAP was not considered necessary. RP was not used because the amplitudes are very weak; SP was not used because it primarily reflects function of the IHCs not the OHCs and it requires approximately 20-

30 dB more intense acoustic stimulus to evoke than CM, meaning it is less sensitive to the stimulus (Cheng, 1994). Finally, CM is commonly used in the clinical setting.

The CM is an alternating potential current (AC) generated by the hair cells in response to acoustic stimuli. It represents the timing and frequency of the stimuli, and the characteristics of its waveform are determined by the acoustic stimuli (Adrian, 1931; Dallos, 1973, 1976; Wever, 1930). In fact, the CM shows a direct phase relationship to the waveform of the acoustic stimulus. When the polarity of the stimulus is changed, there is a reversal of the CM waveform, meaning that the CM follows the waveform of the stimulus. CM is considered a pre-neural response from the cochlea that starts before neural response of ABR peak I, but CM lasts all the time when sound is lasted (Rance, 2005).

The CM is generated primarily by the OHCs. The CM is likely produced by the radial bending or shearing of the stereocilia of the OHCs (Gavara, 2011). If OHCs are destroyed, the CM will be absent; if the auditory nerve is severed, but the OHCs are intact, the CM will be present (Margolis, 1992). Low frequency CM can be measured along the entire basilar membrane, but high frequency CM can be measured only at cochlear basal turns. This fact is because low frequency stimuli can activate the whole basilar membrane, whereas high frequency stimuli can activate the basilar membrane only located at the cochlear basal turns (Margolis, 1992; Patuzzi, 1989a).

CM is measured at different locations in the ear. The invasive technique of round window recording can be used intra-operatively to evaluate the function of hair cells in cochlear implant recipients, but round window recording is not suitable for the routine application in the clinical setting (Choudhury, 2012). A less invasive approach is to measure CM at the mastoid, where the primary electrode is placed on the skin surface of the test side mastoid (Berlin, 1998; Chisin, 1979; Rance, 1999; Sohmer, 1976 and 1980; Starr, 2001). CM was more easily measured by ear canal

recording than by mastoid recording. Also, it was found that the amplitude of CM measured by mastoid recording was small, and the SNR was lower than those recorded by trans-tympanic/ear canal recording, because the distance between the mastoid electrode and the cochlea was longer than that between the trans-tympanic/ear canal electrode and the cochlea (Arslan, 1994 and 1997; Dallos, 1973, 1983; Eggermont, 1976; Johnstone, 1966; Patuzzi, 1989a; Rance, 1999; Sohmer, 1976; Starr, 2001; Withnell, 2001). In other research, it was noted that the amplitude of CM measured by mastoid recording was always smaller than that by ear canal recording (Aran, 1976; Elberling, 1973; Rance, 1999; Starr, 2001; Yoshie, 1969). Thus, researchers concluded that the closer the primary electrode was to the cochlea, the greater the amplitude of CM (Riazi, 2008).

In human research, a customized concha electrode has been designed to record CM, and the CM measured by the concha recording was compared with that by ear canal/mastoid recording. The amplitudes of CM measured by concha recording were higher than that by mastoid recording, but lower than that by ear canal recording (Zhang, 2010).

In summary, the CM can be recorded at several sites, including the ear canal. In the current study, the focus was on comparing measurements made at the round window (CM RW) and the ear canal (CM EC). The ear canal recording of CM is much less invasive, may be superior to mastoid recording, and has great potential in clinical application (Riazi, 2008). However, the relationship between CM RW and CM EC has not been investigated.

1.5.3. Otoacoustic Emissions

In normal hearing there are two important processes: the transmission and amplification of the auditory signal. IHCs transmit auditory signals to the the primary auditory cortices of the brain, and OHCs amplify the auditory signals so that auditory sensitivity and frequency selectivity of

IHC are greatly improved. Therefore, the cochlea is a signal transducer as well as a signal amplifier (Bell, 2006).

The transmission of the auditory signal is an example of mechano-neuroelectrical transduction, in which acoustical/mechanical energy is transformed into neuroelectrical energy via the ion channels located within the stereocilia of hair cells. This neuroelectricity is transmitted to the primary auditory cortices of the brain via the brain stem (Baiduc, 2014; Brownell, 1985).

The amplification of the auditory signal is a reverse transduction process in which the receptor potentials within the hair cells induce mechanical movements of the cells themselves, and the hair cells expand and contract. These mechanical movements can boost the vibrations of the basilar and tectorial membranes so that the auditory signals received by the IHCs are amplified (Baiduc, 2014; Brownell, 1985).

OAE is an indirect evaluation of cochlear function. In mammals, this amplification of the auditory signal is mediated by the electro-motility of the OHCs. When the OHCs are stimulated by the external acoustic sounds, there are alterations in the receptor potentials. In response to the alterations of the receptor potentials, OHCs change their length and shape, which produces mechanical energy. Part of this mechanical energy is converted into acoustic sound, and this sound is transmitted back into middle/outer ear, where it is recorded by a microphone placed in ear canal. These sounds are referred to as evoked otoacoustic emissions (EOAE) (Brownell, 1985 and 1990). When no external acoustic sound is provided to stimulate the OHCs, there is still electrical activity in the OHCs or resting receptor potentials. In this case, OAE is still detectable and is referred to as spontaneous (SOAE) (Baiduc, 2014). The measurement of SOAE requires a highly sensitive probe microphone and very low-noise floor (Penner, 1997; Burns, 1992); neither was feasible in the current study, so SOAE was not used.

EOAE was used in this study. EOAE can be further divided into three classes according to the selection of stimulus, stimulus-frequency (SFOAE), transiently evoked (TEOAE) and distortion product otoacoustic emissions (DPOAE). SFOAEs are signals that have the same frequency as the evoking stimulus. SFOAEs can be used to estimate hearing function, but in practice the differentiation of SFOAEs from the evoking stimulus is challenging because they have the same frequency (John, 2005). TEOAE can be evoked in the range of human primary speech frequency, from 1 kHz to 4 kHz, using an acoustic stimulus about 84 dB SPL in individuals with hearing thresholds of 20 dB HL (Glattke, 2002; Norton, 2000; Kemp, 1978). However, there are two limitations to the use of TEOAE. The first is that if the hearing thresholds at specific frequency exceed 30 dB HL, there would be no TEOAEs typically for that frequency (Glattke, 2002; Harris, 1991, 2002); the second limitation is that when the stimulus frequency is above 5 kHz, there is no substantial TEOAEs in normal hearing adults (Yates, 2000). In this study, it was considered too difficult to measures TEOAE in the noise exposure groups as there would be no substantial TEOAE at 6 kHz and 8 kHz. In summary, based on the study design, neither TEOAE nor SFOAE were used in this study. DPOAE was selected for use in this study as a measure of OHC; it is commonly used in the clinical setting (Glattke, 2002; Harris, 1991, 2002; Norton, 1989).

DPOAE are produced by the OHCs in response to two simultaneous pure-tone stimuli of different frequencies (dual-tone). The stimuli are known as the primary tone f_1, and the secondary tone f_2; f_1 represents the lower frequency stimulus, and f_2 represents the higher frequency stimulus (Hall, 2000).

When the cochlea are presented with dual tones (f_1 and f_2), a mechanical process of non-linear intermodulation between them produces a few new acoustic components of different frequencies along basilar membrane, called distortion products. The frequency of the distortion

product is f_{dp}. There is a relationship between f_1, f_2 and f_{dp}: $f_{dp} = f_1 + N * (f_2 – f_1)$, where N could be any positive/negative integer. The cochlea could produce distortion products of different frequencies; however, the most common distortion product is at the frequency of *2f1-f2*, and this DPOAE is usually used in the clinical setting (Kemp, 1998). DPOAE can be recorded in the ear canal. In a healthy human ear, the recorded signal-to-noise ratio (SNR) of DPOAE could be above 20 dB sound pressure level (SPL). If hair cells are damaged, the DPOAE will be compromised or disappear (Kemp, 1982, 1998). So DPOAE is a sensitive method reflecting the function of OHC.

It is important to note that DPOAE and CM are the tests of OHC function and they are considered indirect hearing tests; specifically, the presence of normal DPOAE and CM does not ensure normal hearing. For example, in auditory neuropathy spectrum disorder, there is neural hearing loss, and ABR would be abnormal or absent, but DPOAE and CM can be normal.

1.6. Previous Related Research

A literature review was conducted on the evaluation of low frequency hearing loss. Only two studies were found in which 0.5 kHz DPOAE were successfully measured (Gorga, 2007; Kiss, 2001). Kiss (2001) did not report how the measurement of 0.5 kHz DPOAE was conducted.

Gorga (2007) measured 0.5 kHz DPOAE in 103 human participants. However, 0.5 kHz DPOAE could not be obtained in all participants' ears, and in some participants, the SNR of 0.5 kHz DPOAE were about 3 dB SPL, which is too low to be considered valid. Also, the strategies they used to obtain the 0.5 kHz DPOAE were effortful; for example, they increased the number of time averages to above 210 so that the background noises were minimized and the SNR of 0.5 kHz DPOAE was maximized. They concluded that without the increased time averages, the high levels of background noises at 0.5 kHz made it impossible to obtain acceptable measurements.

For each stimulus frequency and intensity, the measurement of DPOAE is obtained by averaging a number of 50-ms probe tube microphone waveform. There are two kinds of averaging, time averaging and frequency spectrum averaging (EPL Cochlear Test Suite User Manual). The number of frequency spectrum averages indicates how many frequency spectrum are averaged to obtain the final DPOAE spectrum. The number of time average indicates how many waveform of DPOAE are averaged to obtain the final amplitudes of DPOAE and the final noise floor; the more time averages, the higher the amplitude of DPOAE, and the lower the noise floor.

Although increasing the number of time averages can improve the SNR, too many time averages can produce traces with larger variances that makes the measurements less reliable. Further, from a clinical standpoint, increasing the number of time averages is time-consuming and can cause patient discomfort, making that approach less feasible for use in the clinical setting.

In summary, there is insufficient research on the objective measurement of low frequency hearing function, on the effects of different types of hearing loss on test results, on the morphology and numbers of OHC. Therefore, the current study was conducted to address this lack of information and to extend the knowledge base on these topics.

1.7. Research Questions

1. What are the response patterns of the tests (SNRs of DPOAE and the amplitudes of the ABR and CM) in the presence of normal hearing (Group 1) and NIHL?
2. What are the differences in the three auditory system tests as a function of NIHL, specifically the following:
    a. low frequency band-noise exposure (Group 2)
    b. high frequency band-noise exposure (Group 3)

c. a sequence of low then high frequency band-noise exposure (‘low plus high frequency,’ (Group 4), and,

d. a sequence of high then low frequency band-noise exposure (‘high plus low frequency’ (Group 5)?

3. What is the relationship between the CM measured at the round window and the CM measured at the ear canal in the presence of normal hearing and NIHL?
4. What are the differences in the morphology and number of OHCs as a result of NIHL?

Chapter 2: Method

2.1. Overview of the Experiment

In the first part of the experiment, 15 guinea pigs were randomly allocated to five groups of three animals as follows:

- Group 1, normal control, was not exposed to noise.
- Group 2 was exposed to low frequency band-noises between 0.5~2 kHz (low band-noises).
- Group 3 was exposed to high frequency band-noises between 6~8 kHz (high band-noises).
- Group 4 was exposed to low plus high band-noises.
- Group 5 was exposed to high plus low band-noises.

In each group, the three tests were measured at 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz respectively. The SNR of DPOAE and the amplitudes of ABR and CM were compared between the five groups.

In the second part of the experiment, after the three tests were performed in the five groups, the animals were euthanized and the cochlea were removed for histological study. Silver nitrate staining was adopted, and the animals' basilar membranes were examined under a microscope. Cell morphology and the numbers of missing OHCs were compared between the five groups.

2.2. Procedures

2.2.1. Animals

Fifteen healthy guinea pigs with normal hearing were used in the research. Preyer's reflex was verified in all the animals to prove that the animals had normal hearing. The animals were approximately 2 months old and weighed 250-300 grams. Animals were kept in a quiet room with a 12:12 hour light and dark cycle. The room temperature was controlled at 25° C. Animals were

randomly divided into five groups, each group had 3 animals (6 ears). All animal procedures were reviewed and approved by the *Animal Care and Use Committee*, University of Alberta.

2.2.2. Experimental Instruments

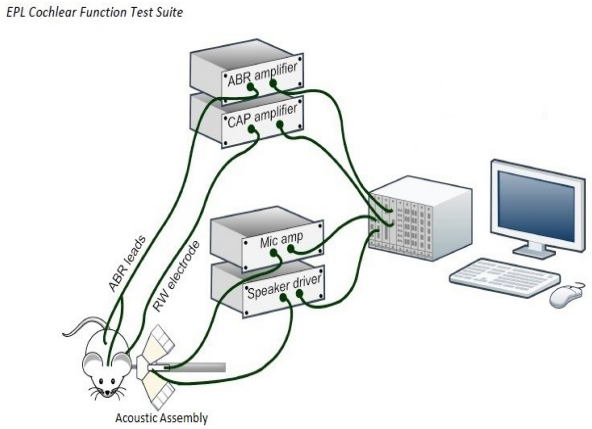
The Eaton-Peabody Laboratories Cochlear Function Test Suite (CFTS) was used in the experiments. The CFTS performs acoustic calibrations and measures cochlear function in animals, such as DPOAE, ABR, and CM. The CFTS is a program written in LabVIEW that generates digital stimuli and acquires response data using National Instruments input/output boards.

Figure 2-1 shows the basic composition of the CFTS. Auditory stimuli were generated digitally by the CFTS software and amplified by an external powered amplifier (2-channel Audio Amp, TDT Stereo Amp & Power Supply, TDT SA-1 (TDT; Tucker Davis Technologies). The amplifier drove two speakers that were housed in the acoustic assembly system. The outputs of the two speakers mixed at the assembly tip, a stainless steel nosepiece. The nosepiece was fitted into the animal ear canal.

A probe tube microphone was coupled with a stainless steel probe tube that could measure the sound pressure at the assembly tip. This microphone was used to calibrate the acoustic outputs from the speakers and to record OAEs generated by the outer hair cells, i.e., the DPOAE (Figure 2-2). The output of the probe-tube microphone was amplified (Mic. Amp, Etymotic Research Microphone Preamp, ER10C) and sent back to an I/O board.

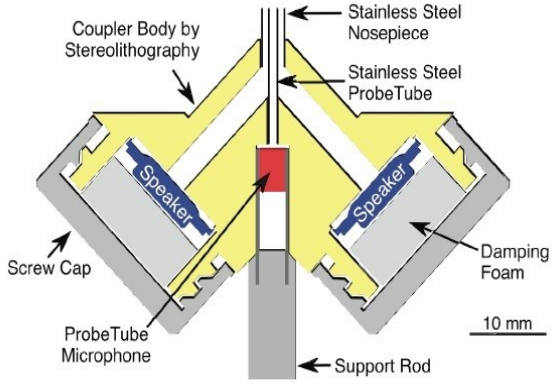
For the recording of ABR, a primary ABR lead was attached to the animal at the vertex. For the recording of the CM EC, a primary electrode was inserted subcutaneously in the ear canal close to the tympanic membrane. For the recording of the CM RW, a customized round window electrode was used. This electrode had a ball-shaped end that was fitted on the round window. The

potentials of ABR and CM were electrical signals. These signals were amplified (Electrode Amp, Grass Instruments Amp & Power Supply, CP-511 & RPS-312) and routed back to the I/O boards.



The CFTS measures cochlear function in animals. The acoustic assembly was fitted into animal ear canal. For the ABR recording, a primary ABR lead was attached to the animal at the vertex. For the recording of CM EC, a primary electrode was inserted in the ear canal. For the recording of CM RW, a primary electrode was placed on the round window.

Figure 2-1. The Schematic Description of the Basic Experimental Setup



There were two systems in the acoustic assembly. One system was the speaker-acoustic-signal delivery system, in which the acoustic signals generated by the speaker were delivered to stimulate the animal ear. Another system was the microphone-acoustic-signal collecting system, in which the acoustic signal generated by the animal cochlea was recorded by the probe tube microphone, (i.e., the DPOAE). Note: the speaker-acoustic-signal delivery system and the microphone-acoustic-signal collecting system were separated.

Figure 2-2. Diagram of the Acoustic Assembly

Before the animal experiments, the intensity of acoustic stimulus was measured and calibrated by a precise sound level meter (¼" Pressure-Field Prepolarized Microphone and Preamplifier, PCB Piezotronics, U.S.A.). The sound level variation was less than 1 dB within the space available to the animal. The laboratory was a non-reverberant room with the background noise level around/below 10 dB SPL. The animal was placed in a sound proof and electrical silent box. Room temperature was controlled at approximately 25 ^{0}C.

2.2.3. Animal Preparation and Operation Procedure

The procedure used in the experiment was similar to that reported previously (Harvey, 1987; Zhang, 1997), as follows:

1. This was an acute experiment, the animal would not wake after experiment and would be euthanized at end of experiment. During the experiment, the animal body temperature was kept by using a 37^0C water heating pad.
2. Anesthesia: a drug combination was available for intramuscular injection, the cocktail of drug combination was a mixture of ketamine 40 mg/kg and DeDomitor 0.15 mg/kg. About 5 min after injection, the surgical plane was reached (Figures 2-3 and 2-4).
3. A single bolus of atropine sulfate 0.05mg/kg was injected subcutaneously to reduce mucosal secretion and the risk of tracheal obstruction. This subcutaneous injection was used to increase the drug release time, so that the serum concentration of atropine would not increase abruptly.
4. The condition of the external ear canal and tympanic membrane (middle ear) was examined to ensure that there were no foreign bodies in the external ear canal, the color and the shape of the tympanic membrane were normal, and there was no perforation in the tympanic membrane.
5. The four tests (DPOAE, ABR, CM RW and CM EC) were measured in the control group; in the noise exposure groups, the tests were measured immediately after noise exposure. For the recording of ABR, the primary active electrode was placed on the vertex, the reference electrode was placed on the mastoid of the test ear, and the ground electrode was placed on the lower back of the animal.

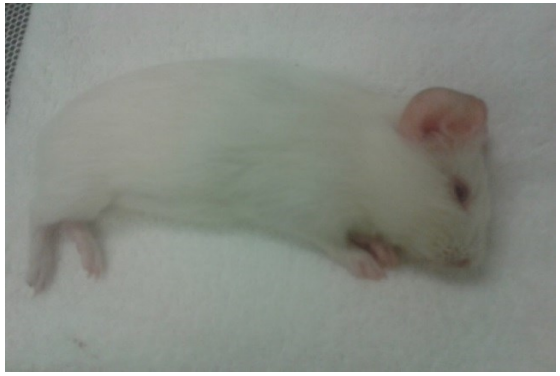
6. For the recording of CM EC, the primary electrode was inserted subcutaneously into the ear canal close to the tympanic membrane. For the recording of the CM RW, an incision was made behind the pinna to expose the bony bulla (Figure 2-4).
7. Before the incision in the skin was made, local anesthesia with 1% lidocaine was injected subcutaneously posterior to the pinna. This was to prevent the rare probability of a very light pain and distress which might occur upon skin incision. The reason for the topical anesthesia was that there was individual variation in the animals' sensitivity to pain stimulus.
8. An electrical drill was used to cut a hole on the bulla (wall of middle ear), so that the access to the round window was available.
9. For the recording of the CM RW, a round window-recording primary electrode was put through the hole and placed on the round window. The round window recording electrode was customized with a ball shaped end to ensure proper fit.
10. The acoustic assembly was fitted into the animal ear canal. There were two important roles of the acoustic assembly. One role was to deliver the acoustic stimulus to the animal ear to evoke hearing test responses, and the other role was to record the DPOAE.
11. Once the above preparation was complete, the delivery of the acoustic stimulation via the speakers, along with recording responses from electrodes and the microphone were started. Each of the four tests was measured at 0.5 kHz, 2 kHz, 4 kHz, 6 kHz and 8 kHz.
12. After the recording was completed, all the attachments (electrodes and probe tube) were removed from the animal.

13. The animal was euthanized by the intra-cardiac injection of potassium chloride (KCl, 1-2 meq/kg or 75-150 mg/kg). After intra-cardiac injection, the heart beat and respiration were checked to make sure the animal was dead.
14. After the animal was dead, the cochlea were removed immediately for histology study. From animal death to the removal of cochlea, the maximum time was no longer than six minutes. Then the histologic study was conducted.

2.2.4. The Generation of NIHL Models

Labview Signal Express (National Instrument) was used to generate the white noise. White noise is a heterogeneous mixture of sound waves extending over a wide frequency range with equal intensities; the frequency range was from 0 - 20 kHz. The noise level was set at 5 Vrms (root mean square voltage) so that the noise output was equal to 120 dB SPL. Band pass filter was set in the Labview signal express. The band pass filter of low frequency noise was set between 0.5~2 kHz (see Figure 2-7) and the band pass filter of high frequency noise was set between 6~8 kHz (see Figure 2-8).

Fifteen animals were randomly allocated into five groups. Group 1 was the normal control, and received no noise exposure. In group 2, animals were exposed to low frequency band-noises at 120 dB for 1 hour. In group 3, animals were exposed to high frequency band-noises at 120 dB for 1 hour. In group 4, animals were exposed to low frequency band-noises at 120 dB for 1 hour. The animals were given a 15 min break and were then exposed to high frequency band-noises at 120 dB for another 1 hour. In group 5, animals were exposed to high frequency band-noises at 120 dB for 1 hour followed by a 15 min break. They were then exposed to low frequency band-noises at 120 dB for another 1 hour.



In the surgical plane of anesthesia there was no toe pinch reflex and no corneal reflex.

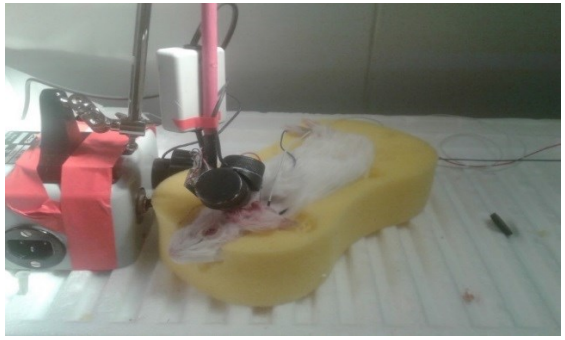
Figure 2-3. Animal at the Surgical Plane of Anesthesia

An incision was made behind the pinna to expose the bony bulla, a hole was drilled on the bulla afterwards to access to the round window.

Figure 2-4. The Incision to Expose the Bony Bulla

During experiment, a cover was put on top of the unit so that the experiments were carried out in a sound proof and electrically silent box.

Figure 2-5. Hearing Testing in Progress



The animal was placed into a customized foam mattress. The dark material on the acoustic assembly is the electrical-magnetic shielding

Figure 2-6. A Close View of the Testing

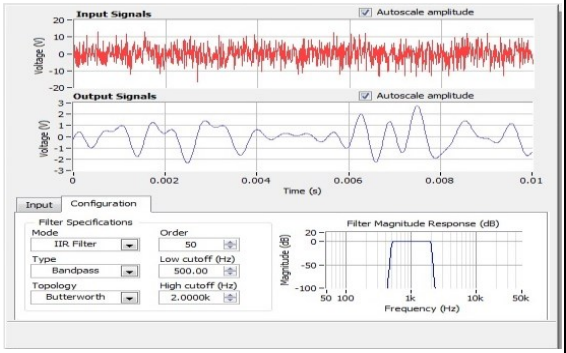


Figure 2-7. The Acoustic Spectrum of Low Frequency Band Noise Exposure

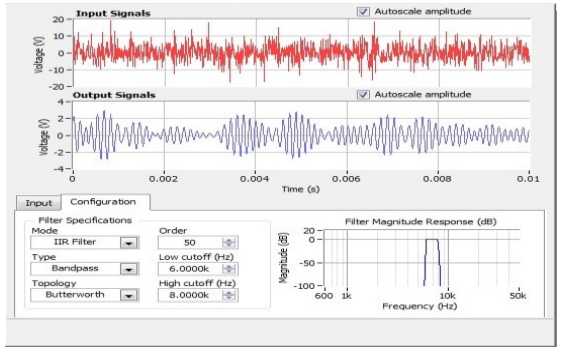


Figure 2-8. The Acoustic Spectrum of High Frequency Band Noise Exposure

2.2.5. Calibration

The system was calibrated to ensure that the auditory stimuli were presented to the animal ears at the set levels. System calibration included three parts, as follows: the calibration of reference microphone, the calibration of probe tube, and in-ear calibration. The calibration of reference microphone involved the sensitivity of the reference microphone, with a correct reference microphone used later in the calibration of probe tube.

The calibration of probe tube was conducted within the acoustic assembly to calculate the exact relationship between the SPL at the end of the probe tube and the voltage out of the acoustic assembly microphone. This calibration was made by measuring the SPL near the tip of the probe-tube with the reference microphone, while simultaneously measuring the voltage out of the acoustic assembly microphone. The probe tube calibration was performed by holding the reference microphone at a very short distance from the end of the probe tube using a calibration coupler. The probe tube calibration used the ratio of the output of the acoustic assembly microphone to the output of the reference microphone, so the SPL of the acoustic stimuli used for the calibration was cancelled out.

To do the probe tube calibration, a chirp (a brief sound that contained all frequencies throughout the range to be calibrated) was produced by one of the earphone speakers, and the outputs of the acoustic assembly microphone and the reference microphone were simultaneously measured. Then, the test software could compute the ratio of the voltage out of the acoustic-assembly microphone to the SPL at the end of the probe tube.

Finally, the in-ear calibration was performed right before the measurement of DPOAE. In-ear calibration involved computing the ratio of the voltage applied to the earphone speakers to the SPL at the end of the probe tube (near the tympanic membrane of the animal).

2.2.6. Optimizing the Recordings

Two important modifications were made on the experimental instruments to optimize recording. First, the probe tube assembly was painted with CuPro-Cote conductive copper-bearing paint, and the painting was done three times. This electromagnetic shielding was to reduce the stimulus pickup by the recording electrodes. The second modification was a sealing tube fitted onto the acoustic assembly tip (nosepiece). This sealing tube was used to reduce environmental interruption, such as background noise.

2.2.6.1. Optimizing the Recordings of 0.5 kHz DPOAE

The first step to ensure the successful measurement of 0.5 kHz DPOAE was to ensure the appropriate stimuli parameters, as the appropriate parameters could help to evoke the most robust responses that best reflected cochlear status. Two pure tones (*f1* and *f2*) were presented simultaneously to an animal ear to evoke DPOAE. The *f1* was the primary tone, and *f2* was the secondary tone. Three stimulus-related parameters needed to be selected before the measurements of 0.5 kHz DPOAE, specifically, the *f1* level, the range of separation between *f1* and *f2* level, and the frequency ratio between *f1* and *f2*.

The f1 level. The DPOAE generated by the moderate *f1* level between 55-65 dB sound pressure level (SPL), is useful in clinical differential diagnoses. Either higher or lower *f1* levels could cause errors in the diagnosis of hearing loss. Higher *f1* levels could result in underestimation of hearing loss, and lower *f1* levels could overestimate the hearing loss; when the *f1* is low, the SNR is reduced, which makes DPOAE less reliable, and people with normal hearing could be mistakenly classified as having hearing loss (Stover, 1996).

In small mammalian animals, like guinea pigs, the DPOAE evoked by very high *f1* levels do not reflect cochlear function; the SNRs of DPOAE evoked by very low *f1* level are small; thus, the

f1 level between 55 and 70 dB SPL was the best choice (Mills, 1996; Whitehead, 1992a and b). In the current study, the *f1* level was set at 70 dB SPL.

Range of separation between the f1 and f2 levels. In previous research, when the *f1* level was set at a moderate value, a 10 dB separation between *f1* and *f2* level could evoke the most robust responses of DPOAE, and yield the largest amplitude of DPOAE (Gaskill, 1990; Popelka, 1993). In the current study, the *f1* level was set at 70 dB SPL and the *f2* level was set at 60 dB SPL.

Frequency ratio between f1 and f2. When the ratio of *f2*/*f1* was 1.2, the most robust response of DPOAE could be evoked (Abdala, 1996; Gaskill, 1990). As the optimal frequency ratio of *f2*/*f1* seems to be 1.2, in the current study, *f2*/*f1* was set at 1.2, which meant the frequency of the secondary tone (*f2*) was presented at 1.2 times higher than the frequency of the primary tone (*f1*); the five *f1* frequencies used in the study were: 0.6 kHz, 2.4 kHz, 4.8 kHz, 7.2 kHz, 9.6 kHz; the five *f2* frequencies used in the study were: 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, 8 kHz.

After the stimuli parameters were optimized, there were four fundamental steps in the measurements of DPOAE. Before anesthesia, all the animals were tested by Preyer's reflex. The researcher stood behind the animals, clapped hands, and observed the animals' responses. At the sound of the clapping, the expected response was ears upwards and claws withdrawn. A positive Preyer's reflex meant the integrity of animal hearing pathway, including the conducting function of middle ear.

Otoscopies were performed on all animals under a surgical microscope. Otoscopy was conducted to ensure the canals were clean of debris, cerumen, etc. that could obstruct the probe tube and interfere with measurements of DPOAE. The tympanic membrane was also examined by otoscopy, to examine the color and the shape of the tympanic membrane, and to make sure the membrane was normal, with no perforation.

To ensure stability and minimize body movement during all tests, the animal was placed in a customized foam mattress and the posture of animal ear canal was fixed. If the ear canal was sealed well, the interruption from the background noise would be reduced, and the SNR of DPOAE would be increased. To further minimize the interruption from background noises, a small elastic plastic tube was fitted onto the probe tube. The far end of the plastic tube was trimmed into a slope shape, so that the animal ear canal was sealed tight.

2.2.6.2. Optimizing the Recording of Low Frequency ABR

ABR is useful in the evaluation of hearing function, however there are challenges that must be addressed in the recording of 0.5 kHz ABR. The first is the presence of low synchronous activity at 0.5 kHz. The amplitude of ABR is dependent on the extent of neural synchrony, and the higher stimulus frequency, the more neural synchronies exist. When 0.5 kHz tone burst is used to evoke ABR, a partition close to the cochlear apex is stimulated. However, the cochlea has less synchronous activity in this partition, so the signal of ABR evoked by 0.5 kHz tone burst is weak (Jerger, 1978).

The second challenge in the recording of 0.5 kHz ABR is the interruption of background electrical noise. The 0.5 kHz ABR is vulnerable to the interruption of background electrical noise, and in this experiment, there were two sources of the electrical noise: one was from the electrophysiological activity of the animal, and the other was from the influence of external electrical device, such as computers, amplifiers, and filters (Marcoux, 2012). Further, the sedation drug (ketamine) used in the anesthetic cocktail could minimize muscular activity so that electrophysiological activity from the animal itself was reduced. If possible, the electrical devices were placed at a distance from the animal. When the electrical devices in the testing environment

could not be moved away from the animal, the electromagnetic shielding helped to reduce the interference of extraneous noises.

The third challenge in the recording of 0.5 kHz ABR was the interruption of background acoustic noise. At low frequencies between 0.07 and 1 kHz, background acoustic noises could cause delay in the ABR waveform and reduce its amplitude (Timing, 2011). The level of environmental acoustic noise at low frequency is usually higher than that at high frequency. In the current study, ABR was recoded in a customized sound proof box to reduce the interference of acoustic noise.

Environmental electrical activity and/or background noises are common interruptions to the recording electrodes. In this study, the acoustic assembly was painted with electrical-magnetic shielding to minimize these interruptions. Remaining interruptions were cancelled out by setting up the frequency filters in the Grass instruments, such that only the ABR signal would remain and be amplified. Low and equal impedance at sites of the electrode placements was also an important factor for the recording of ABR (Campbell, 1993, 1994 and 2012). Before each experiment, impedance was checked, and was maintained around 0.5 kΩ; the stimulus repetition rate was 27/sec.

2.2.6.3. Optimizing the Recording of Low Frequency CM

Similar to the recording of ABR, low frequency CM, particularly the 0.5 kHz CM, is vulnerable to the electromagnetic interference (Zhang, 2010; 2013). In addition to the electrical-magnetic shielding on the acoustic assembly, additional modifications were made for the recording of low frequency CM.

The rate of stimulus repetition was set at 30/second. As CM is a pre-neural response, it is not vulnerable to neural fatigue and it may be better that the recording is as fast as the time window permits. This rate of stimulus repetition could reduce the acquisition time of response signals and avoid neural fatigue. The time window of the response signal was set at 8.5 milliseconds. Because the actual time window of valid CM signals was very short, the signals of valid CM would stop long before 8.5 milliseconds. This short time window of CM allowed the rapid rate of stimulus repetition to be used, so that the cochlear partition that was corresponding to the stimulus frequency could be investigated well before neural fatigue occurred.

The signals of 0.5 kHz CM are vulnerable to environmental interruption, such as myogenic and electrical activity of the brain (Ferraro, 2006). The recorded 0.5 kHz CM was amplified by the Grass instrument, and two filters were set in the Grass instrument: the low pass filter was set at 0.3 kHz, and the high pass filter was set at 1 kHz. Only the signals between 0.3 and 1 kHz were passed onto the computer for further analysis, so the signals of 0.5 kHz CM were purified, and environmental interruption could be minimized.

All the recorded signals of CM were sinusoidal, specifically, they had mirror images in the two stimulus polarity waveforms. The signals of CM began within 1 millisecond of stimulus onset, and lasted up to 5 or 6 milliseconds. It was critical to verify that the recorded signals were not neural responses, and the recorded signals were not stimulus artifacts.

The recorded signals were potentials. To prove that the measured potentials were not neural responses, the stimulus polarity was alternated, then the polarity of the measured potential was reversed. This alternation and reversal supported that the measured potentials were CM, not neural responses. If the response polarity had not reversed with the changes in the stimulus polarity, then the measured potentials would be neural responses, not CM.

To prove that the measured potentials were not stimulus artefacts, a pilot experiment of tube blocking was performed before the recording of CM. In the acoustic assembly, there were two remote speakers coupled by a probe tube. The probe tube tip was fitted into animal ear canal. This distance between the remote speakers and the probe tube tip could introduce a time delay between the electrical signals applied on the speakers and the acoustic stimuli at the ear canal. The time delay enabled the separation of stimulus artefact from the measured CM. The stimulus artefacts were like random electromagnetic signals. In the pilot experiment of tube blocking, the probe tube was blocked by plastic clay, and the acoustic stimuli could not be delivered to the animal ear canal. This pilot work was an indispensable part of the test procedure; in this condition the stimulus artefacts from the speakers remained, but the animals could not receive the acoustic stimuli, so no CM response was expected. The recorded signals were the artefacts only and served as an important negative control. In the study following the pilot experiment, the plastic clay was removed from the probe tube, and the measured CM response could be validated or rejected according to the negative control of artefact. The onset of artefact always occurred before the onset of CM.

2.2.7. The Calculation of the Response Magnitude of the Three Hearing Tests

For ABR and the CM, the intensity of the acoustic stimulus was at 70 dB SPL. For the DPOAE, the intensity of *f1* stimulus ranged from 35 to 72.5 dB SPL. When the *f1* intensity reached at 70 dB SPL, the amplitudes of DPOAE were at the stage of plateau, and the data were considered appropriate for statistical analysis.

The amplitudes of ABR were calculated as the peak-to-peak amplitude of the positive value of the most dominant wave, usually wave V, and the negative value of the next trough (Neil, 1998; Popelar, 2008; Walger, 1993), as illustrated in Figure 2-9. The amplitudes of CM were measured

as the difference between the peak of a given polarity and the peak of the opposite polarity. Three peak-to-peak cycles around the midpoint of the 3.5-msec period were selected, and the amplitudes of the three cycles were averaged (Zhang, 2012a), as illustrated in Figure 2-10. The SNR of DPOAE were calculated as the plateau amplitudes of DPOAE minus noise floor (Lyons, 2004), as shown in Figure 2-11.

The DPOAE input/output function (I/O function) is created by plotting the measured SNR of DPOAE as a function of the f_2 level. The DPOAE iso-response (DP-gram) is created by comparing each I/O function to find out the levels of L_2 that produce a criterion SNR value; and these L_2 levels are then plotted as a function of f_2 level. A family of such iso-response is established by changing the criterion SNR values. Because the primary analyses in this study involved comparison of SNR between the five groups, not comparison of L_2 level between the five groups, the DP-gram was not used.

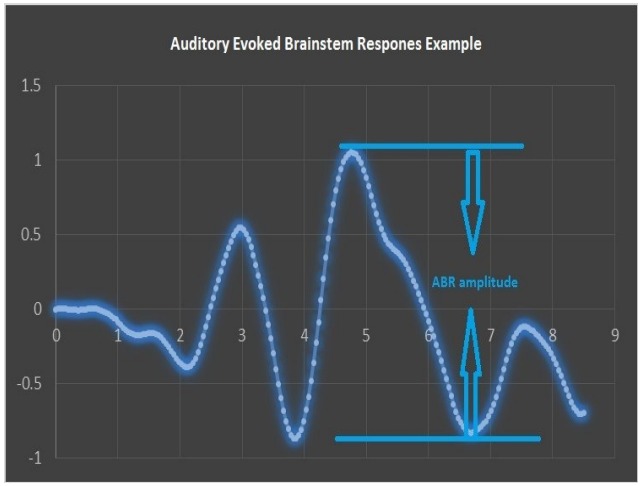
After the SNR of DPOAE and the amplitudes of ABR/CM were calculated in the five groups, the data were compared between the five groups, the response patterns of hearing tests were plotted, and the relationship between CM RW and CM EC was investigated.

In this study, the tests were conducted at supra-threshold levels. Threshold testing was not conducted for two reasons. First, the necessary equipment was not available for use in the current study. Second, and more importantly, there are inconsistencies between behavioural thresholds and hearing function with each of the tests. For ABR thresholds, at middle frequencies between 2 kHz to 4 kHz, in adults with normal hearing, ABR threshold tests seem to be less sensitive than behavioral thresholds, with a maximum difference up to 20 dB (Stapells, 2000a, 2000b and 2002); for patients with sensorineural hearing loss, the difference between ABR threshold tests and behavioral thresholds is up to 15 dB (Gorga, 2002; Stapells, 2000a, 2000b). The major part of this

estimation error is due to unknown factors that are involved in the physiological property of the two tests, so this large estimation error is not controllable (Van, 1987). Furthermore, in mammalian animals the ABR threshold diverges significantly from the behavioral thresholds at the low and high frequency ranges (Heffner, 2003). In the current study, the frequency range was from 0.5 kHz to 8 kHz, and the focus of the research was on low frequency hearing function; thus, ABR thresholds were not used.

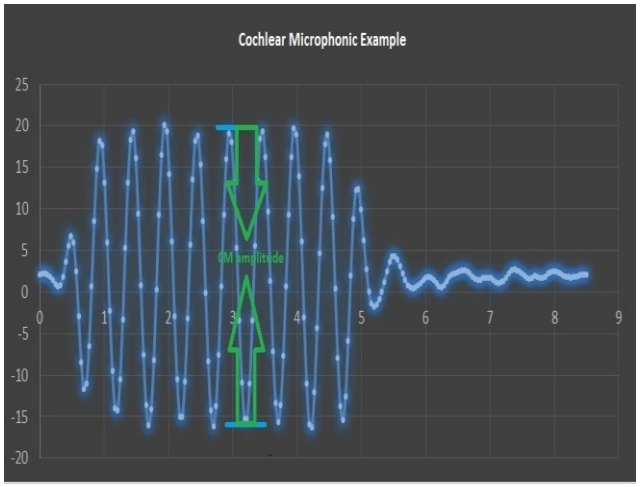
With regard to DPOAE thresholds, it was reported that best correlation between DP thresholds and behavioral thresholds was at 4 kHz; correlations decreased as frequency either increases or decreases and DP threshold could not be used to predict behavioral thresholds at low frequency ranges (Gorga, 2003). Thus, DP thresholds were not used in the current study in which the focus was on low frequency hearing function. Likewise with CM, as stated in the *Guidelines for Cochlear Microphonic Testing* (Lightfoot, 2011), the CM threshold is not a useful predictor of behavioural thresholds.

In summary, the fundamental aims of the research - the optimization of the recording technology of three tests, investigation of their feasibility for the evaluation of hearing function, particularly at low frequencies, and study of test sensitivity for changes in the hearing function before and after noise exposure – could be met by the current study design and measures employed.



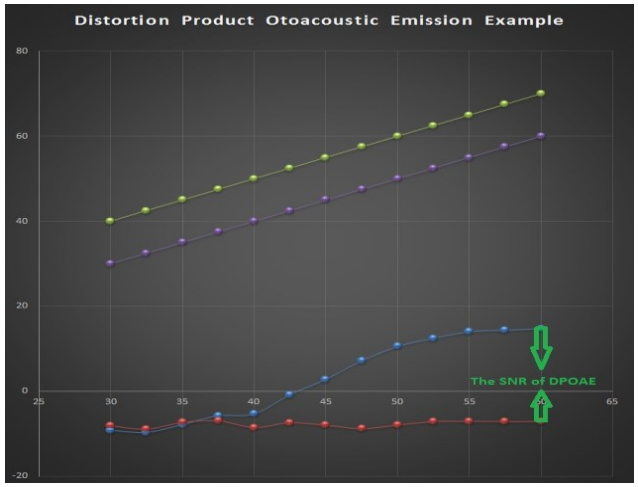
This is a waveform example of the ABR. The unit of x axis was millisecond, and the unit of y axis was microvolt. The ABR amplitudes were calculated as the peak-to-peak amplitude of the positive value of the most dominant wave, usually wave V, and the negative value of the next trough.

Figure 2-9. Calculating the Amplitudes of ABR



This is a waveform example of the CM. The unit of the x axis was millisecond and the unit of the y axis was micro-voltage (microvolt). The CM amplitude was measured as the difference between the maximum peak of a given polarity and the maximum peak of the opposite polarity.

Figure 2-10. Calculating the Amplitudes of CM



This is a waveform example of the DPOAE. The blue line indicates the DPOAE amplitude. The red line indicates the noise floor. The DPOAE SNR was calculated as the DPOAE plateau amplitude minus noise floor. There were two acoustic stimuli for DPOAE, *f1* and *f2*. The light green line indicates the *f1* level, L1; the purple line indicates the *f2* level, L2; L1=L2+10. The unit of the x and y axes is dB SPL, and the x axis represents the L2 level.

Figure 2-11. Calculating the SNR of DPOAE

2.2.8. The Histology Study

Once hearing tests were completed, and all attachments (electrodes and acoustic assembly) were removed from the animal, the animal was euthanized, and the two cochlea from each animal were removed for silver nitrate staining.

Silver nitrate staining was used to label the hair cells and stereocilia as described in previous research (Li, 2010). After each cochlea was removed, a small hole was made at the cochlear apex with a 27-gauge needle, and the round window was opened as well. A 0.5% solution of silver nitrate in distilled water was perfused through the round window three times. The cochlea was perfused with distilled water followed by 10% formalin (pH 7.2), three times, and then immersed in the fixative solution for 24 h. The basilar membrane was dissected out, trimmed and mounted in glycerin on a glass slide as a flat surface preparation. Slides were exposed to sunlight for approximately 1 h to enhance the brownish-black staining of the stereocilia. Specimens were examined under a light microscope at 400X.

Single-blind counting was adopted for this analysis. The specimen label was covered, so that the group number was unknown when the specimen was examined. The morphology of hair cells in Group 1 was used as the normal control, and the noise exposure groups were compared with group 1 to examine any changes in the morphology of OHCs (swelling and loss of steriocilia). The full basilar membrane was examined, and the number of missing OHCs was counted. The data were compared between the five groups.

2.3. Statistical Analysis

A bio-statistician consulted on all statistical analyses. To answer research question 1 (*What are the response patterns of the tests in the presence of normal hearing (Group 1) and NIHL?*), the response patterns of the DPOAE (in SNR), ABR and CM were analyzed across the five test frequencies (0.5 kHz, 2 kHz, 4 kHz, 6 kHz and 8 kHz) in the five test groups (Group 1 – normal control, Groups 2-5, different types of NIHL). In each group there were three animals (six ears), thus, at each of the five frequencies there were six measurements for each test. At each of the measured frequencies, the SNRs of DPOAE and the amplitudes of ABR and CM were averaged, and the means were plotted against the measured frequencies.

To answer research question 2 (*What are the differences in the three tests as a function of NIHL?*), at each of the measured frequencies, repeated measures ANOVA were used to compare the mean SNRs or the amplitudes within the five groups. The dependent variables were the SNRs or the amplitudes of hearing tests, and the independent variable was noise exposure (with five levels). The SNR or the amplitudes were also compared between the five groups, in mixed ANOVA, and the number of unique comparisons was 10 (4+3+2+1=10). The post hoc Bonferroni correction was performed for each analysis.

The Bonferroni correction can be conducted in two ways that are mathematically equivalent. The first method is through modification of the desired alpha-level and the second is through adjustment of the *p*-value by the numbers of the unique comparisons. In this study, the ANOVA was calculated in SPSS, and the *p*-value after the post-hoc Bonferroni correction was re-calculated by the *p*-value before the Bonferroni correction times the numbers of the unique comparisons

(http://www-01.ibm.com/support/docview.wss?uid=swg21476685). In each pairwise comparison, p <.05 was used to indicate statistical significance. Although this Bonferroni correction was appropriate to use to control for increased rates of Type I errors associated with multiple ANOVA tests, it did not control for family wise increase in error rates associated with other statistical tests (i.e., correlations and one-way ANOVAs used to answer research questions 3 and 4). Further, adjusting the statistical significance by the numbers of unique comparisons can produce more issues than it solves; the main concern is that the interpretation of a test result is affected by the number of other tests which are carried out at the same time. The other concern is that the real significant differences are considered non-significant because the likelihood of Type II errors increases (Perneger, 1998).

To answer research question 3 (*What is the relationship between the CM measured at the round window and the CM measured at the ear canal in the presence of normal hearing and NIHL?*) a Pearson Product Moment Correlation Coefficient was conducted, with a two tailed test of significance ($p < 0.05$) selected.

To answer research question 4 (*What are the differences in the morphology and number of OHCs as a result of NIHL?*) the numbers of missing OHCs were counted in each cochlea (6 per group) and an average was computed for each group. One way between groups ANOVA was used to compare the average numbers of missing OHCs between the five groups. Post-hoc Bonferroni correction was performed. For all the comparisons, p <.05 was taken to indicate statistical significance. All statistical analyses were completed using SPSS programs (IBM SPSS Statistics 19).

It is important to discuss the sample size in this study. Because of large effect sizes, and based on a power analysis conducted with the biostatistician, sufficient statistical power was available to detect differences between and within groups if such differences existed. Also, it is common to have a small sample size in animal experiments. The sample size of 3 animals (6 ears) per group was used in previous research, specifically in Subramaniam (1994), Wang (1998), Gary (2004), and Tona (2014).

Although two ears in the same animal are functionally related, for the purposes of statistical analysis, the ears may be considered independent of each other. In the mammalian brain, there are two auditory cortices, right and left, and it is known that each auditory cortex has independent selectivity of auditory signals (Merzenich, 1985; Schreiner, 1990). Considering the potential interaction between each animal's two ears, repeated measures ANOVA was used. In repeated measures ANOVA, the within subject effect test involved testing of the independence of each animal's two ears. There was no interaction between each animal's two ears, demonstrating independence of the two ears for the purposes of statistical analyses.

Chapter 3: Results

3.1. Research Question 1: What are the response patterns of the tests in the presence of normal hearing and NIHL?

The overall responses of an auditory function test across all measured frequencies is called a response pattern. The response pattern reflects the standard characteristics of the normal cochlea; in the groups exposed to the band-noises, the changes in the responses patterns provided a general estimation of hearing across all frequencies.

3.1.1. The Response Pattern of DPOAE

The means and standard deviations of the SNRs in the five groups are presented in Table 3-1.

Table 3-1: The Mean SNR of DPOAE in the Five Groups (Mean ± SD, group n = 6 ears)

	0.5 kHz	2.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
1: N	21.55 ± 1.21	25.65 ± 1.29	26.02 ± 1.59	27.61 ± 1.50	27.53 ± 1.30
2: L	17.23 ± 1.48	19.71 ± 1.33	24.36 ± 1.36	26.52 ± 1.65	27.04 ± 1.33
3: H	21.38 ± 1.30	25.39 ± 1.27	25.65 ± 1.10	21.26 ± 1.31	20.76 ± 1.38
4: L + H	16.34 ± 1.24	19.36 ± 1.47	24.15 ± 1.37	20.53 ± 1.48	20.62 ± 1.34
5: H + L	16.66 ±1.24	19.49 ± 1.46	24.19 ± 1.41	20.44 ± 1.32	20.31 ± 1.26

N - normal control; L – exposure to low frequency band-noise; H - exposure to high frequency band-noise; (L+H) exposure to low plus high frequency band-noises; (H+L) exposure to high plus low frequency band-noises. The SNR of DPOAE was measured in dB SPL.

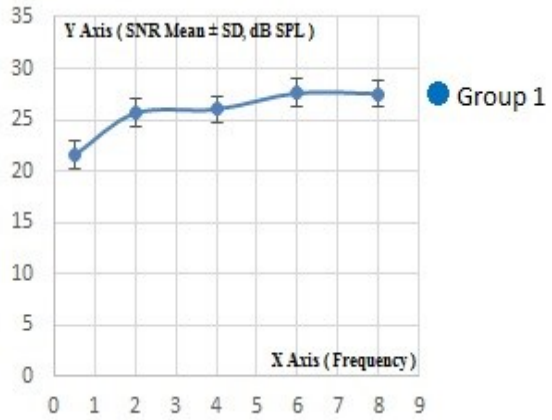
Figure 3-1 shows the response patterns of DPOAE in group 1, the lowest SNR of the DPOAE was at 0.5 kHz, the highest was at 6 kHz. With the increment of frequency, the SNR of DPOAE

had a tendency to increase. The response pattern of DPOAE shown in Figure 3-1 may represent the standard characteristics of the normal DPOAE.

Animals in group 2 were exposed to low frequency band-noises (0.5 kHz-2 kHz). In figure 3-2, reductions in the SNR of 0.5/2 kHz DPOAE were observed in Group 2, as compared to group 1. At all the other measured frequencies, there was little difference in the response pattern between groups 2 and 1.

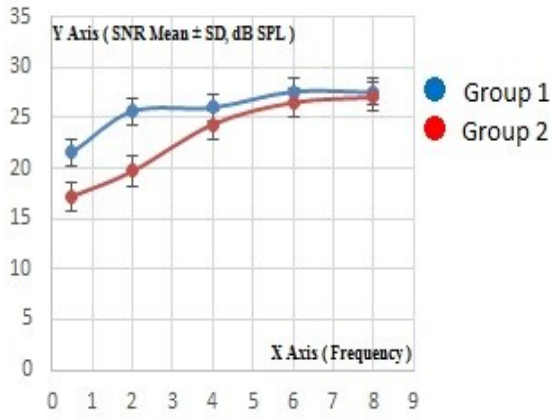
Group 3 was exposed to high frequency band-noises (6 kHz-8 kHz). In Figure 3-3, the SNRs at 6 kHz and 8 kHz in group 3 were lower than their counterparts in group 1; at all the other measured frequencies, there were no differences in the response patterns between the two groups.

Group 4 was exposed to low plus high frequency band-noises (0.5 kHz-2 kHz and 6 kHz-8 kHz); and group 5 was exposed to high plus low frequency band-noises (6 kHz-8 kHz and 0.5 kHz-2 kHz). In Figures 3-4 and 3-5, at 0.5 kHz, 2 kHz, 6 kHz and 8 kHz, reductions in the SNR were observed in groups 4 and group 5, as compared to group 1. At 4 kHz, there was no change in the response patterns in groups 4 and 5.



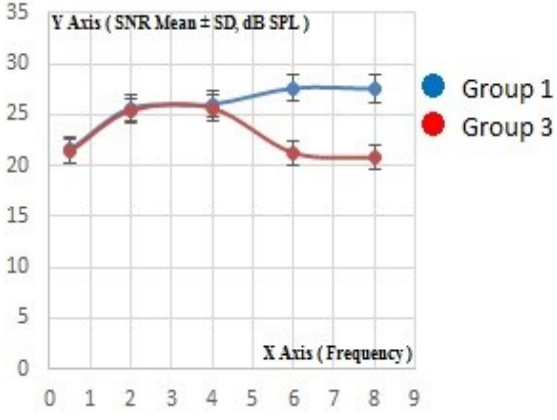
X axis - measured frequencies, in kHz
Y axis – SNR of DPOAE in dB SPL

Figure 3-1. The Response Pattern of DPOAE in Group 1



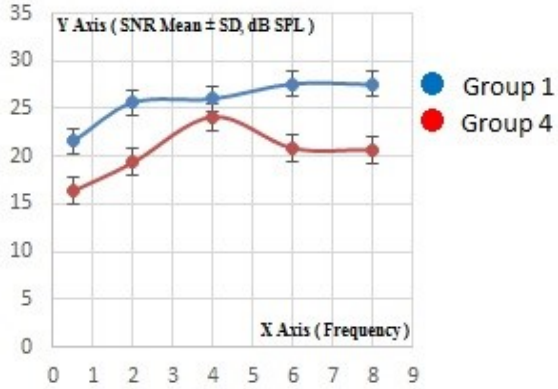
X axis - measured frequencies, in kHz
Y axis – SNR of DPOAE in dB SPL

Figure 3-2. The Response Pattern of DPOAE in Group 1 and Group 2



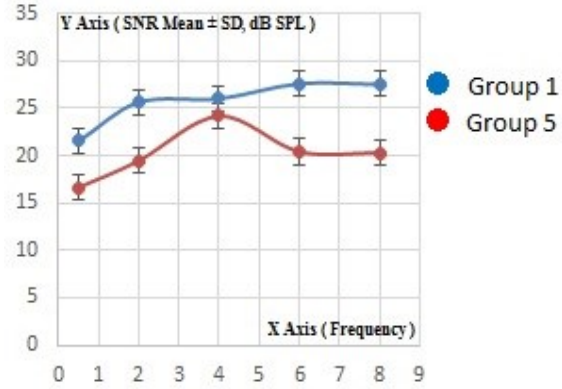
X axis - measured frequencies, in kHz
Y axis – SNR of DPOAE in dB SPL

Figure 3-3. The Response Pattern of DPOAE in Group 1 and Group 3



X axis - measured frequencies, in kHz
Y axis – SNR of DPOAE in dB SPL

Figure 3-4. The Response Pattern of DPOAE in Group 1 and Group 4



X axis - measured frequencies, in kHz
Y axis – SNR of DPOAE in dB SPL

Figure 3-5. The Response Pattern of DPOAE in Group 1 and Group 5

3.1.2. The Response Pattern of ABR

The means and standard deviations of the amplitudes in the five groups are presented in Table 3-2. Figure 3-6 shows the response pattern of ABR in Group 1. The lowest amplitude of the ABR was at 0.5 kHz, the highest amplitude of ABR was at 8 kHz. In group 1, with the increment of frequency, the amplitude of ABR had a tendency to increase. The response pattern of ABR shown in group 1 may represent the standard characteristics of the normal ABR.

Table 3-2: The Mean Amplitudes of ABR in the Five Groups (Mean ± SD, group n = 6 ears)

	0.5 kHz	2.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
1: N	1.91 ± 0.25	2.43 ± 0.35	2.65 ± 0.27	2.87 ± 0.30	2.90 ± 0.33
2: L	0.98 ± 0.31	1.47 ± 0.31	2.54 ± 0.31	2.72 ± 0.35	2.79 ± 0.29
3: H	1.78 ± 0.36	2.28 ± 0.36	2.57 ± 0.39	1.81 ± 0.31	1.76 ±0.34
4: L + H	0.89 ± 0.35	1.43 ± 0.34	2.51 ± 0.41	1.76 ± 0.33	1.72 ± 0.37
5: H + L	0.90 ± 0.34	1.46 ± 0.38	2.51 ± 0.35	1.75 ± 0.34	1.71 ± 0.35

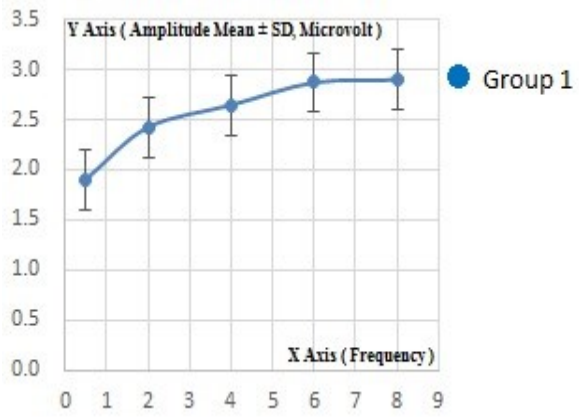
N - normal control; L – exposure to low frequency band-noise; H - exposure to high frequency band-noise; (L+H) exposure to low plus high frequency band-noises; (H+L) exposure to high plus low frequency band-noises. The amplitude of ABR was measured in microvolt

Group 2 was exposed to low frequency band-noises. In figure 3-7, at 0.5 kHz and 2 kHz reductions in the amplitudes of ABR were observed in group 2 compared to group 1. At other measured frequencies, there were no differences in the response patterns of ABR between groups 2 and 1.

Group 3 was exposed to high frequency band-noises. In Figure 3-8, at 6 kHz and 8 kHz reductions in the amplitudes of ABR were observed in group 3 as compared to group 1. At other

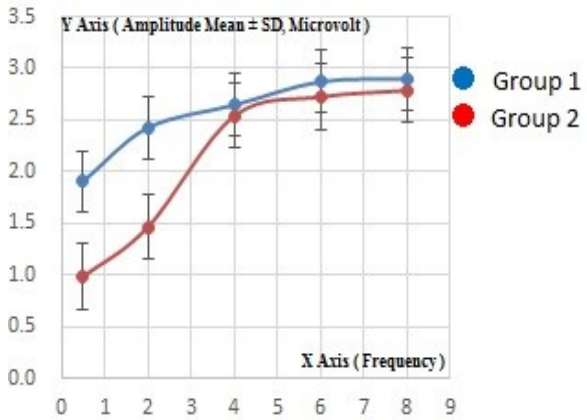
measured frequencies, there were no differences in the response patterns of ABR between groups 3 and 1.

Group 4 was exposed to low plus high frequency band-noises, and group 5 was exposed to high plus low frequency band-noises. In Figures 3-9 and 3-10, at 0.5 kHz, 2 kHz, 6 kHz and 8 kHz reductions in the amplitudes of ABR were observed in groups 4 and 5 compared to group 1 respectively. At 4 kHz, there was no changes in the response patterns of ABR in groups 4 and 5.



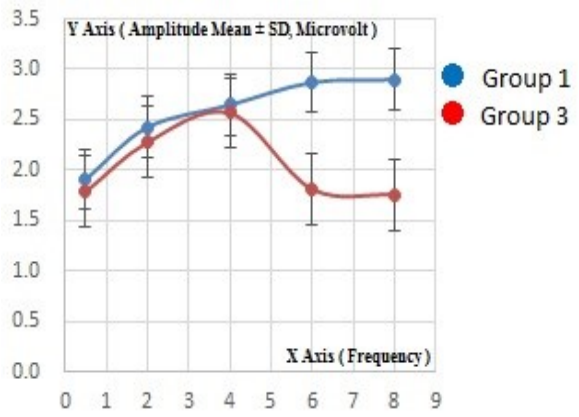
X axis - measured frequencies, in kHz
Y axis – amplitudes of ABR in microvolt

Figure 3-6. The Response Pattern of ABR in Group 1



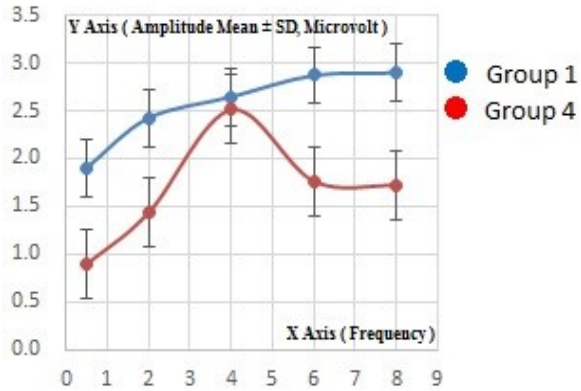
X axis - measured frequencies, in kHz
Y axis – amplitudes of ABR in microvolt

Figure 3-7. The Response Pattern of ABR in Group 1 and Group 2



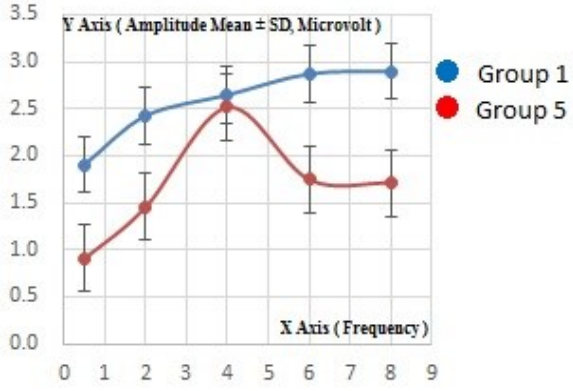
X axis - measured frequencies, in kHz
Y axis – amplitudes of ABR in microvolt

Figure 3-8. The Response Pattern of ABR in Group 1 and Group 3



X axis - measured frequencies, in kHz
Y axis – amplitudes of ABR in microvolt

Figure 3-9. The Response Pattern of ABR in Group 1 and Group 4



X axis - measured frequencies, in kHz
Y axis – amplitudes of ABR in microvolt

Figure 3-10. The Response Pattern of ABR in Group 1 and Group 5

3.1.3. The Response Pattern of CM

The means and standard deviations of CM RW and CM EC amplitudes in the five groups are presented in Tables 3-3 and 3-4, respectively.

Table 3-3: The Mean Amplitudes of CM RW in the Five Groups (Mean ± SD, group n = 6 ears).

	0.5 kHz	2.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
1: N	48.58 ± 1.13	37.35 ± 1.35	34.54 ± 1.36	29.73 ± 1.36	17.01 ± 1.53
2: L	20.88 ± 1.55	20.82 ± 1.72	33.16 ± 1.50	27.06 ± 1.94	15.89 ± 1.92
3: H	46.45 ± 2.24	35.26 ± 2.27	33.50 ± 1.24	17.80 ± 2.20	10.47 ± 1.37
4: L + H	19.93 ± 1.35	20.13 ± 1.48	32.81 ± 1.50	17.44 ± 1.51	9.96 ± 1.49
5: H + L	20.40 ± 1.36	20.55 ± 1.36	33.00 ± 1.63	17.26 ± 1.78	9.89 ± 1.45

N - normal control; L – exposure to low frequency band-noise; H - exposure to high frequency band-noise; (L+H) exposure to low plus high frequency band-noises; (H+L) exposure to high plus low frequency band-noises. The amplitude of CM RW was measured in microvolts.

Table 3-4: The Mean Amplitudes of CM EC in the Five Groups (Mean ± SD, group n = 6 ears).

	0.5 kHz	2.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
1: N	5.11 ± 0.31	4.10 ± 0.32	3.58 ± 0.31	3.11 ± 0.36	2.04 ± 0.35
2: L	2.17 ± 0.32	2.03 ± 0.24	3.43 ± 0.35	2.86 ± 0.33	1.89 ± 0.22
3: H	4.81 ± 0.29	3.93 ± 0.22	3.44 ± 0.31	1.36 ± 0.27	0.65 ± 0.23
4: L + H	2.09 ± 0.31	1.95 ± 0.22	3.38 ± 0.31	1.32 ± 0.26	0.64 ± 0.22
5: H + L	2.12 ± 0.31	1.98 ± 0.23	3.40 ± 0.32	1.30 ± 0.26	0.63 ± 0.22

N - normal control; L – exposure to low frequency band-noise; H - exposure to high frequency band-noise; (L+H) exposure to low plus high frequency band-noises; (H+L) exposure to high plus low frequency band-noises. The amplitude of CM EC was measured in microvolts.

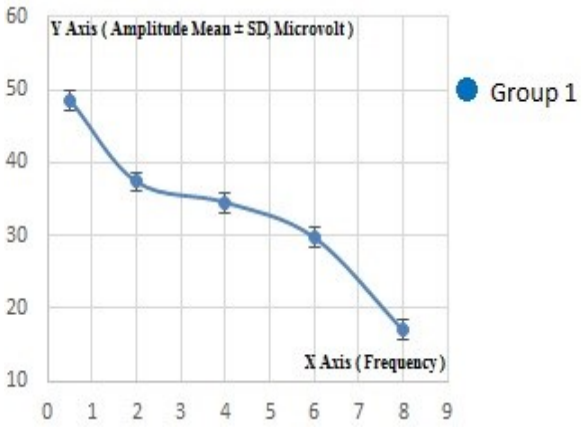
Figures 3-11 and 3-16 show the response pattern of CM RW and CM EC, respectively, in group 1. The highest amplitude of CM was at 0.5 kHz, and the lowest amplitude was at 8 kHz. In group 1, with the increment of frequency, the amplitudes of CM had a tendency to decrease. Group 1 was the normal control, and as such, the response pattern of CM shown in group 1 may reflect the standard characteristics of the normal CM.

Figures 3-12 and 3-17 shows the response pattern of CM RW and CM EC, respectively, in group 2. At 0.5 kHz and 2 kHz, reductions in the amplitudes of CM were observed in group 2. At other measured frequencies, there were no differences in the response patterns of CM between groups 2 and 1.

Figures 3-13 and 3-18 show the response pattern of CM RW and CM EC, respectively, in group 3. At 6 kHz and 8 kHz, reductions in the amplitudes of CM were observed in group 3. At other measured frequencies, there were no differences in the response patterns of CM between groups 3 and 1.

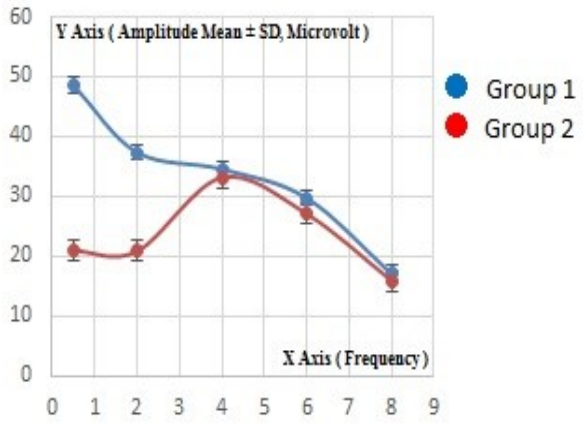
Figures 3-14 and 3-19 show the response pattern of CM RW and CM EC, respectively, in group 4. Figures 3-15 and 3-20 show the response pattern of CM RW and CM EC, respectively, in group 5. At 0.5 kHz, 2 kHz, 6 kHz and 8 kHz, reductions in the amplitudes of CM were observed in groups 4 and 5. At 4 kHz, there were no changes in the response patterns of CM.

In summary, comparison between the response patterns of hearing tests in group 1 and those in the groups exposed to band-noises provides a direct illustration of frequency specific hearing loss induced by noise exposure.



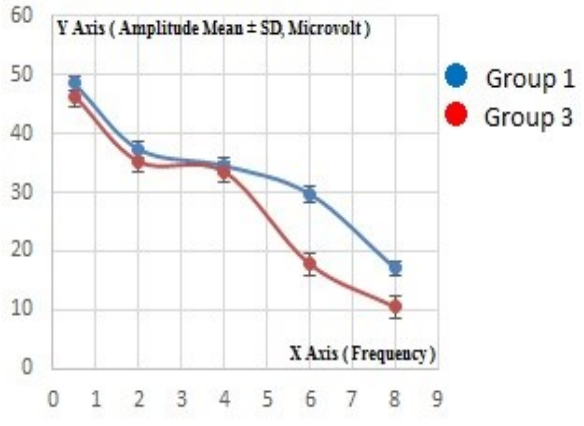
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM RW in microvolt

Figure 3-11. The Response Pattern of CM RW in Group 1



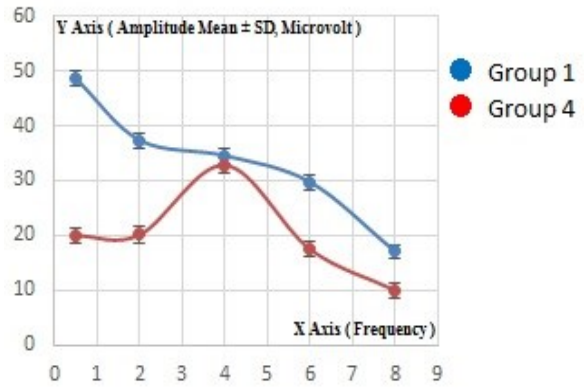
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM RW in microvolt

Figure 3-12. The Response Pattern of CM RW in Group 1 and Group 2



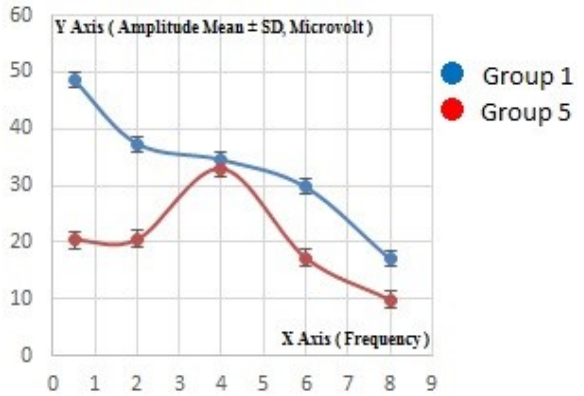
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM RW in microvolt

Figure 3-13. The Response Pattern of CM RW in Group 1 and Group 3



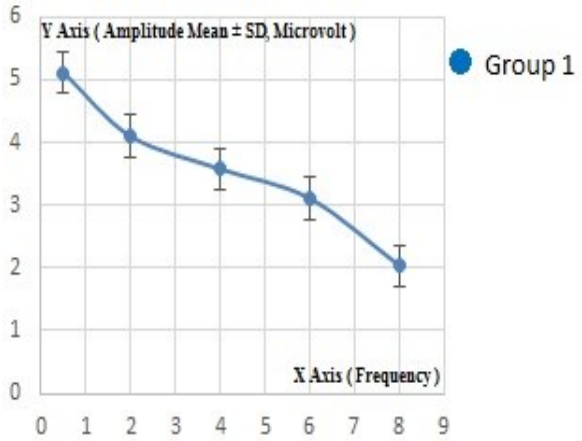
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM RW in microvolt

Figure 3-14. The Response Pattern of CM RW in Group 1 and Group 4



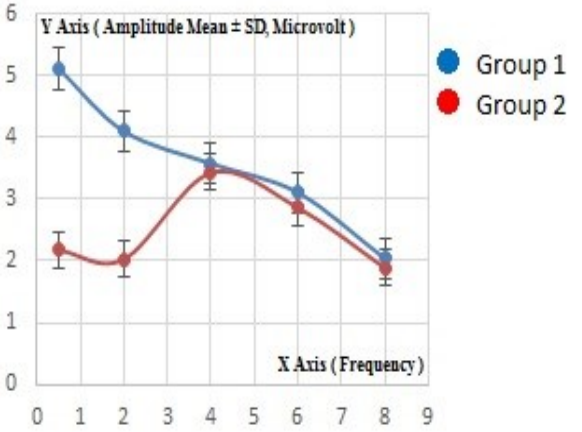
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM RW in microvolt

Figure 3-15. The Response Pattern of CM RW in Group 1 and Group 5



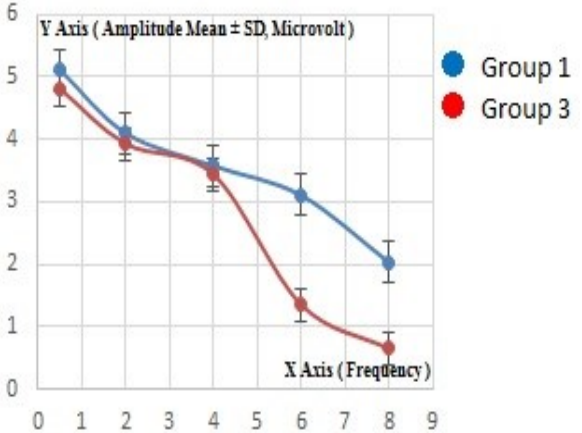
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM EC in microvolt

Figure 3-16. The Response Pattern of CM EC in Group 1



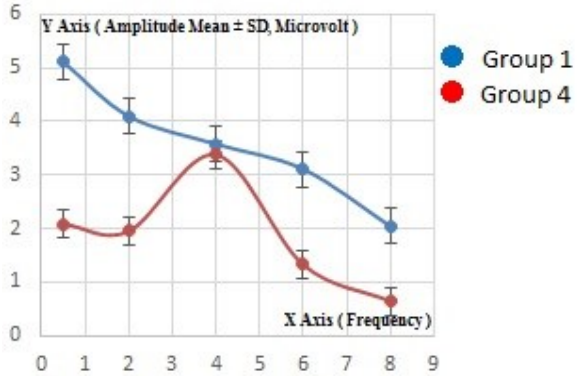
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM EC in microvolt

Figure 3-17. The Response Pattern of CM EC in Group 1 and Group 2



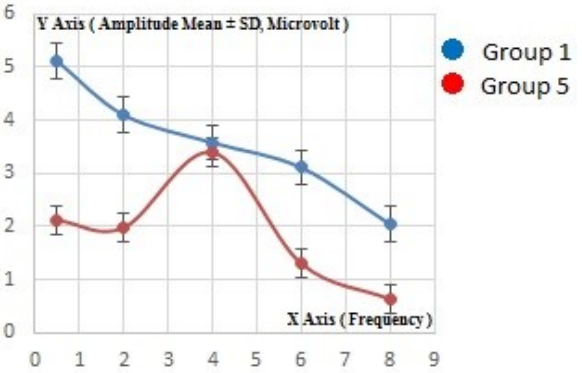
X axis - measured frequencies, in kHz
Y axis – amplitudes of CM EC in microvolt

Figure 3-18. The Response Pattern of CM EC in Group 1 and Group 3



X axis - measured frequencies, in kHz
Y axis – amplitudes of CM EC in microvolt

Figure 3-19. The Response Pattern of CM EC in Group 1 and Group 4



X axis - measured frequencies, in kHz
Y axis – amplitudes of CM EC in microvolt

Figure 3-20. The Response Pattern of CM EC in Group 1 and Group 5

3.2. Research Question 2 – What are the Differences in the Results of the Three Tests as a Function of NIHL?

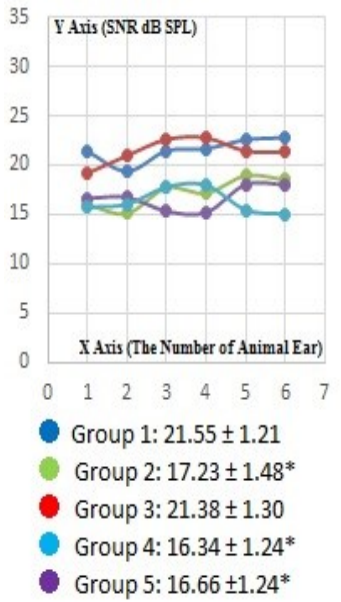
3.2.1. The Changes of DPOAE as a Function of NIHL

DPOAE was measured at five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz and 8 kHz in the five groups (Table 3-5). At each of the measured frequencies, a figure was developed which contained five curves, with each curve representing a group. There were five figures for the five frequency sections (see Figures 3-21 through 3-25).

Table 3-5: The SNR of DPOAE in the Five Groups

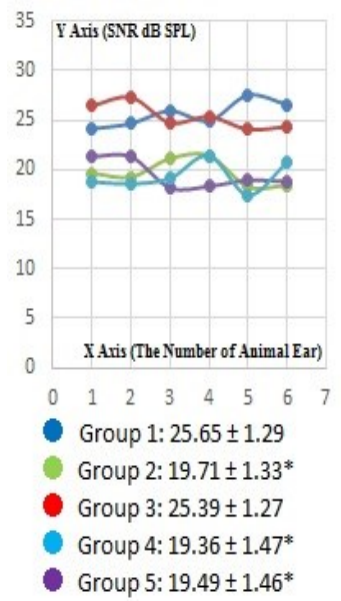
	Ear No.	1	2	3	4	5	6	Mean	SD
0.5 kHz	Group 1	21.41	19.38	21.46	21.67	22.58	22.78	21.55	1.21
	Group 2*	15.97	15.10	17.69	17.15	18.94	18.51	17.23	1.48
	Group 3	19.18	20.96	22.61	22.77	21.44	21.31	21.38	1.30
	Group 4*	15.74	16.03	17.78	17.98	15.46	15.03	16.34	1.24
	Group 5*	16.64	16.73	15.33	15.18	18.03	18.03	16.66	1.24
2 kHz	Group 1	24.14	24.68	25.95	24.96	27.54	26.61	25.65	1.29
	Group 2*	19.67	19.27	21.14	21.44	18.31	18.43	19.71	1.33
	Group 3	26.46	27.32	24.75	25.37	24.09	24.35	25.39	1.27
	Group 4*	18.79	18.60	19.13	21.44	17.44	20.73	19.36	1.47
	Group 5*	21.35	21.32	18.19	18.33	18.94	18.80	19.49	1.46
4 kHz	Group 1	26.55	26.47	24.02	24.08	27.46	27.56	26.02	1.59
	Group 2	22.82	24.48	22.79	24.39	25.88	25.79	24.36	1.36
	Group 3	25.83	26.87	26.89	25.40	24.48	24.42	25.65	1.10
	Group 4	23.99	24.57	25.58	25.59	22.42	22.73	24.15	1.37
	Group 5	24.11	24.26	22.57	22.66	25.76	25.78	24.19	1.41
6 kHz	Group 1	28.08	28.35	25.77	25.66	28.89	28.88	27.61	1.50
	Group 2	24.47	24.36	27.23	27.55	27.58	27.93	26.52	1.65
	Group 3*	22.05	21.63	19.43	19.79	22.25	22.43	21.26	1.31
	Group 4*	19.21	21.89	22.11	18.72	19.76	21.51	20.53	1.48
	Group 5*	20.98	20.57	21.76	21.66	18.89	18.76	20.44	1.32
8 kHz	Group 1	28.92	28.59	25.97	25.88	27.89	27.94	27.53	1.30
	Group 2	27.88	27.96	27.81	27.95	25.37	25.27	27.04	1.33
	Group 3*	22.15	22.77	19.74	19.34	20.38	20.18	20.76	1.38
	Group 4*	20.26	20.13	19.66	22.74	21.72	19.23	20.62	1.34
	Group 5*	19.47	19.39	21.89	21.98	19.56	19.55	20.31	1.26

At 0.5 kHz and 2 kHz, * indicates statistically significant differences from group 1 and group 3, $p<.05$; there were no significant differences between group 1 and 3, and among groups 2, 4 and 5, $p>.05$. At 4 kHz, there were no significant differences among the five groups, $p>.05$. At 6 kHz and 8 kHz, * indicates significantly different from group 1 and group 2, $p<.05$; there were no significant differences between group 1 and 2, and among groups 3, 4 and 5, $p>.05$. The SNR of DPOAE were in dB SPL.



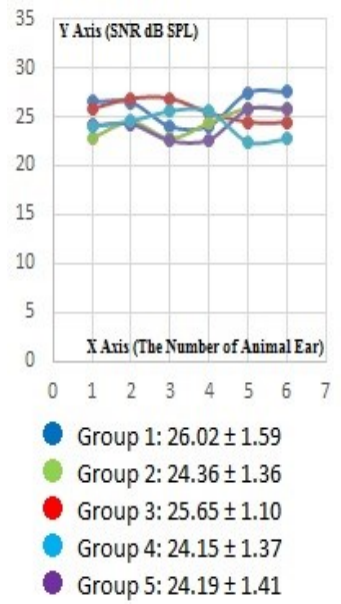
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$=10.835, df=4). * indicates significant reduction in the SNR of 0.5 kHz DPOAE were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-21. The SNR of 0.5 kHz DPOAE in the Five Groups



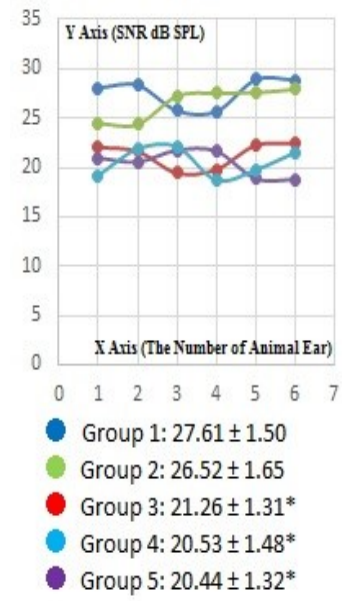
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$= 17.692, df=4). * indicates significant reduction in the SNR of 2 kHz DPOAE were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-22. The SNR of 2 kHz DPOAE in the Five Groups



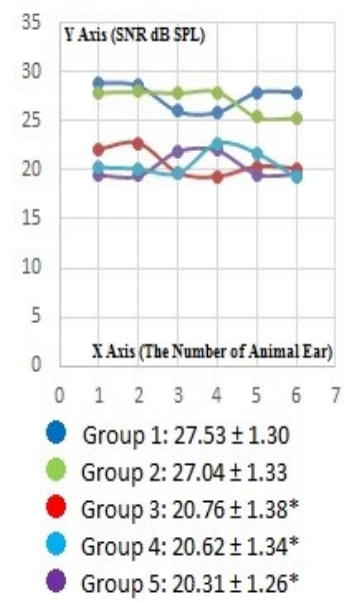
ANOVA showed there were no main group effects between the five groups, ($F_{(4, 10)}$= 1.120, df=4). At 4 kHz, there were no significant differences in the SNR of 4 kHz DPOAE between the five groups, $p>.05$.

Figure 3-23. The SNR of the 4 kHz DPOAE in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$= 17.565, df=4). *indicates significant reduction in the SNR of 6 kHz DPOAE were found in group 3, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-24. The SNR of 6 kHz DPOAE in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$= 22.953, df=4). * indicates significant reduction in the SNR of 8 kHz DPOAE were found in group 3, 4 and 5, as compared to group 1; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-25. The SNR of 8 kHz DPOAE in the Five Groups

X axis - the number of animal ears; y axis - the SNR of DPOAE in dB SPL.
The dark blue curve was group 1, the green was group 2, the red was group 3, the light blue was group 4, and the purple was group 5.

The SNR of DPOAE in the five groups (Mean ± SD, group n = 6 ears) are shown in Table 3-5. The ANOVA showed main group effects (0.5 kHz and 2 kHz, $F_{(4,10)}$ were 10.84 and 17.69 respectively, $p<0.01$). To examine specific group differences, Bonferroni post hoc analysis were conducted. In terms of the SNR of 0.5 kHz and 2 kHz DPOAE, there were no significant differences between groups 1 and group 3, $p>0.05$ (0.5 kHz $F_{(1,10)} = 0.02$; 2 kHz $F_{(1,10)} = 0.05$). In terms of the SNR of 0.5 kHz and 2 kHz DPOAE, no significant differences were observed between group 2, 4 and 5, $p>0.05$ {(0.5 kHz between G2 and G4 $F_{(1,10)} = 0.63$; between G2 and G5 $F_{(1,10)} = 0.26$; between G4 and G5 $F_{(1,10)} = 0.08$) (2 kHz between G2 and G4 $F_{(1,10)} = 0.10$; between G2 and G5 $F_{(1,10)} = 0.04$; between G4 and G5 $F_{(1,10)} = 0.01$)}.

The SNR of 0.5 kHz and 2 kHz DPOAE in both group 1 or group 3 was significantly higher than that in group 2, 4, or 5 respectively, $p<0.05$ {(0.5 kHz between G1 and G2 $F_{(1,10)} = 14.88$; between G1 and G4 $F_{(1,10)} = 21.64$; between G1 and G5 $F_{(1,10)} = 19.06$; between G3 and G2 $F_{(1,10)} = 13.74$; between G3 and G4 $F_{(1,10)} = 20.26$; between G3 and G5 $F_{(1,10)} = 17.77$) (2 kHz between G1 and G2 $F_{(1,10)} = 28.80$; between G1 and G4 $F_{(1,10)} = 32.34$; between G1 and G5 $F_{(1,10)} = 30.99$; between G3 and G2 $F_{(1,10)} = 26.36$; between G3 and G4 $F_{(1,10)} = 29.76$; between G3 and G5 $F_{(1,10)} = 28.46$)}.

These results indicated that significant reduction in the SNR of 0.5 kHz and 2 kHz DPOAE was observed as a function of low frequency band noise, or the combination of low and high frequency band noises. However, the reduction in the SNR of 0.5 kHz and 2 kHz DPOAE caused by the combination of low and high frequency band noises was not significantly higher than that caused by single low frequency band noise.

At 4 kHz no significant group main effect was found ($F_{(4,10)}$ was 4.78, $p>0.05$). Bonferroni post hoc analysis showed that there was no significant difference in the SNR of 4 kHz DPOAE between the five groups, $p>0.05$ (between G1 and G2 $F_{(1,10)} = 1.95$; between G1 and G3 $F_{(1,10)} = 0.099$; between G1 and G4 $F_{(1,10)} = 2.48$; between G1 and G5 $F_{(1,10)} = 2.36$; between G2 and G3 $F_{(1,10)} = 1.17$; between G2 and G4 $F_{(1,10)} = 0.03$; between G2 and G5 $F_{(1,10)} = 0.02$; between G3 and G4 $F_{(1,10)} = 1.59$; between G3 and G5 $F_{(1,10)} = 1.50$; between G4 and G5 $F_{(1,10)} = 0.00$).

At 6 kHz and 8 kHz there were main group effects. ($F_{(4,10)}$ were 17.57 and 22.96 respectively, $p<0.01$). Bonferroni post hoc analysis showed that in terms of the SNR of 6 kHz and 8 kHz DPOAE, there were no significant differences between group 1 and group 2, $p>0.05$ (6 kHz $F_{(1,10)} = 0.86$; 8 kHz $F_{(1,10)} = 0.20$). In terms of the SNR of 6 kHz and 8 kHz DPOAE, no significant differences were observed between groups 3, 4 and 5, $p>0.05$ {(6 kHz between G3 and G4 $F_{(1,10)} = 0.38$; between G3 and G5 $F_{(1,10)} = 0.49$; between G4 and G5 $F_{(1,10)} = 0.00$) (8 kHz between G3 and G4 $F_{(1,10)} = 0.01$; between G3 and G5 $F_{(1,10)} = 0.17$; between G4 and G5 $F_{(1,10)} = 0.08$)}.

However, in terms of the SNR of 6 kHz and 8 kHz DPOAE, both group 1 and group 2 were significantly higher than groups 3, 4, or 5 respectively, $p<0.05$ {(6 kHz between G1 and G3 $F_{(1,10)} = 28.89$; between G1 and G4 $F_{(1,10)} = 35.93$; between G1 and G5 $F_{(1,10)} = 36.92$; between G2 and G3 $F_{(1,10)} = 19.85$; between G2 and G4 $F_{(1,10)} = 25.75$; between G2 and G5 $F_{(1,10)} = 26.59$) (8 kHz between G1 and G3 $F_{(1,10)} = 38.65$; between G1 and G4 $F_{(1,10)} = 40.23$; between G1 and G5 $F_{(1,10)} = 44.00$; between G2 and G3 $F_{(1,10)} = 33.24$; between G2 and G4 $F_{(1,10)} = 34.71$; between G2 and G5 $F_{(1,10)} = 38.21$)}. ***This finding indicated that high frequency band noise, or the combination***

of high plus low frequency band noises, could cause significant reduction in the SNR of 6 kHz and 8 kHz DPOAE.

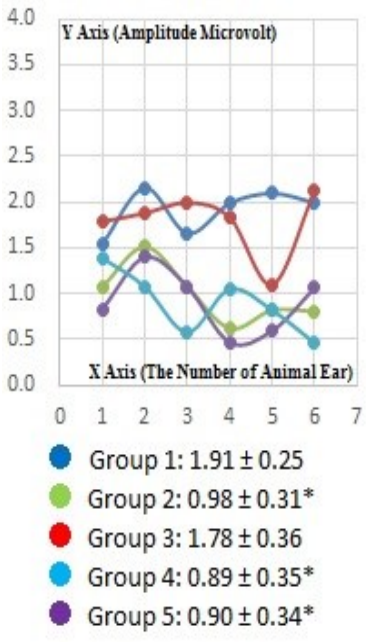
3.2.2. The Changes of ABR as a Function of NIHL

The amplitudes of ABR in the five groups are shown in Table 3-6. Five figures were developed according to the five measured frequencies (see Figures 3-26 through 3-30).

Table 3-6: The Amplitudes of ABR in the Five Groups

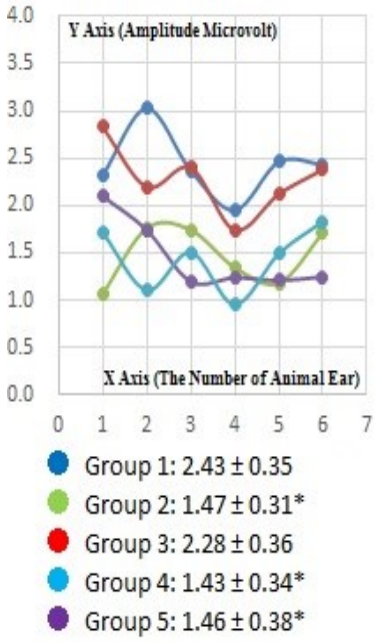
	Ear No.	1	2	3	4	5	6	Mean	SD
0.5 kHz	Group 1	1.55	2.16	1.66	1.99	2.09	1.99	1.91	0.25
	Group 2*	1.08	1.51	1.07	0.62	0.82	0.81	0.98	0.31
	Group 3	1.79	1.88	1.99	1.83	1.10	2.12	1.78	0.36
	Group 4*	1.39	1.08	0.58	1.05	0.82	0.46	0.89	0.35
	Group 5*	0.83	1.40	1.08	0.47	0.59	1.06	0.90	0.34
2 kHz	Group 1	2.32	3.02	2.37	1.95	2.46	2.43	2.43	0.35
	Group 2*	1.06	1.76	1.74	1.34	1.18	1.71	1.47	0.31
	Group 3	2.83	2.19	2.40	1.74	2.12	2.38	2.28	0.36
	Group 4*	1.72	1.11	1.51	0.95	1.49	1.83	1.43	0.34
	Group 5*	2.10	1.74	1.20	1.24	1.21	1.25	1.46	0.38
4 kHz	Group 1	2.35	3.09	2.62	2.38	2.74	2.69	2.65	0.27
	Group 2	2.18	2.76	2.53	2.85	2.15	2.77	2.54	0.31
	Group 3	2.15	2.85	2.67	3.02	2.69	2.03	2.57	0.39
	Group 4	1.87	3.13	2.65	2.30	2.58	2.54	2.51	0.41
	Group 5	1.99	2.50	2.40	2.53	3.09	2.58	2.51	0.35
6 kHz	Group 1	2.51	3.34	2.59	2.84	2.96	2.98	2.87	0.30
	Group 2	2.32	2.46	3.31	2.81	2.82	2.61	2.72	0.35
	Group 3*	1.37	1.78	1.70	2.32	1.81	1.88	1.81	0.31
	Group 4*	1.78	1.83	2.07	1.12	1.87	1.92	1.76	0.33
	Group 5*	1.77	2.09	1.10	1.86	1.94	1.73	1.75	0.34
8 kHz	Group 1	2.47	3.43	2.64	2.85	2.97	3.03	2.90	0.33
	Group 2	3.26	2.52	2.75	2.85	2.90	2.44	2.79	0.29
	Group 3*	1.94	1.75	1.79	2.09	1.10	1.86	1.76	0.34
	Group 4*	1.64	1.66	2.13	1.07	1.85	1.97	1.72	0.37
	Group 5*	1.67	2.10	1.08	1.84	1.93	1.65	1.71	0.35

At 0.5 kHz and 2 kHz, * indicates significantly different from group 1 and group 3, $P<.05$; there were no significant differences between group 1 and 3, and among group 2, 4 and 5, $P>.05$. At 4 kHz, there were no significant differences among the five groups, $P>.05$. At 6 kHz and 8 kHz, * indicates significantly different from group 1 and group 2, $P<.05$; there were no significant differences between group 1 and 2, and among group 3, 4 and 5, $P>.05$. The amplitudes of ABR were in microvolt.



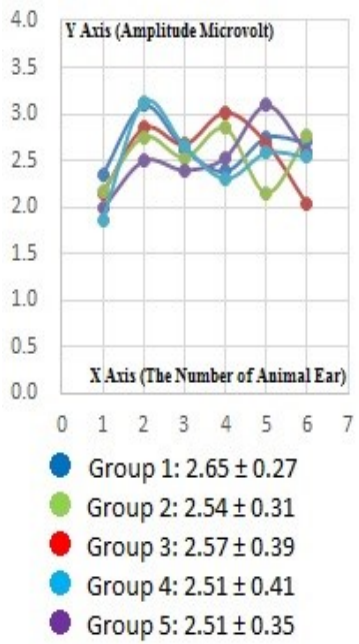
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$= *16.035, df=4*). * indicates significant reduction in the amplitudes of 0.5 kHz ABR were found in group 2, 4 and 5, as compared to group 1, *p<.05*; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, *p>.05*.

Figure 3-26. The Amplitudes of 0.5 kHz ABR in the Five Groups



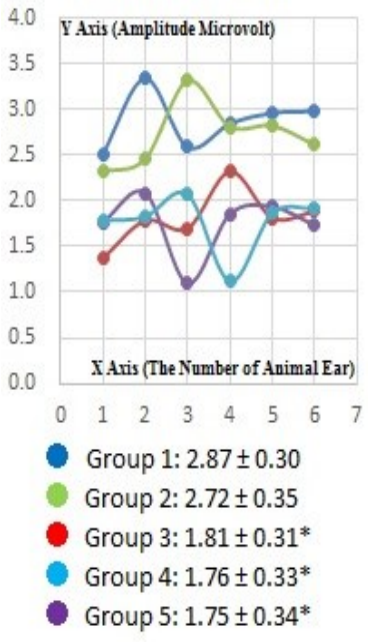
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$ = *11.195, df=4*). * indicates significant reduction in the amplitudes of 2 kHz ABR were found in group 2, 4 and 5, as compared to group 1, *p<.05*; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, *p>.05*.

Figure 3-27. The Amplitudes of 2 kHz ABR in the Five Groups



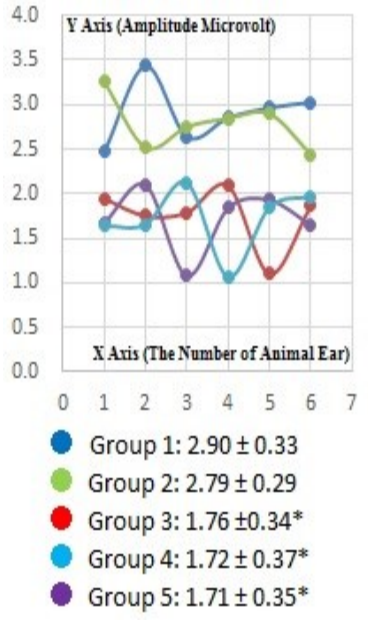
ANOVA showed there were no main group effects between the five groups, ($F_{(4, 10)}$ = *.246, df=4*). The dark blue curve was group 1, the green curve was group 2, the red was group 3, the light blue was group 4, and the purple was group 5. At 4 kHz, there were no significant differences between the five groups, *p>.05*.

Figure 3-28. The Amplitudes of 4 kHz ABR in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$ = *18.344, df=4*). * indicates significant reduction in the amplitudes of 6 kHz ABR were found in group 3, 4 and 5, as compared to group 1, *p<.05*; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, *p>.05*.

Figure 3-29. The Amplitudes of 6 kHz ABR in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)}$ = *33.715, df=4*). * indicates significant reduction in the amplitudes of 8 kHz ABR were found in group 3, 4 and 5, as compared to group 1, *p<.05*; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, *p>.05*.

Figure 3-30. The Amplitudes of 8 kHz ABR in the Five Groups

X axis - the number of animal ear, y axis - the amplitudes of ABR in microvolt.
The dark blue curve was group 1, the green was group 2, red was group 3, light blue was group 4, purple was group 5.

There were variations in the amplitudes of ABR between the five groups. The highest amplitude of ABR was seen in the normal group, and the amplitude of ABR was reduced after noise exposure. The variations in the amplitudes of ABR between the five groups were analyzed using repeated measures ANOVA.

At 0.5 kHz and 2 kHz, main group effects existed ($F_{(4,10)}$ were 16.03 and 11.20 respectively, $p<0.01$). To examine specific group differences, Bonferroni post hoc analysis were conducted. In term of the amplitudes of 0.5 kHz and 2 kHz ABR, there were no significant differences between groups 1 and group 3, $p>0.05$ (0.5 kHz $F_{(1,10)} = 0.46$; 2 kHz $F_{(1,10)} = 0.50$). In terms of the amplitudes of 0.5 and 2 kHz ABR, there was no significant difference between groups 2, 4 and 5, $p>0.05$ {(0.5 kHz between G2 and G4 $F_{(1,10)} = 0.25$; between G2 and G5 $F_{(1,10)} = 0.20$; between G4 and G5 $F_{(1,10)} = 0.00$) (2 kHz between G2 and G4 $F_{(1,10)} = 0.02$; between G2 and G5 $F_{(1,10)} = 0.00$; between G4 and G5 $F_{(1,10)} = 0.01$)}.

The amplitudes of 0.5 kHz and 2 kHz ABR in both group 1 and group 3 were significantly higher than those in groups 2, 4, or 5 respectively, $p<0.05$ {(0.5 kHz between G1 and G2 $F_{(1,10)} = 26.63$; between G1 and G4 $F_{(1,10)} = 32.09$; between G1 and G5 $F_{(1,10)} = 31.45$; between G3 and G2 $F_{(1,10)} = 20.09$; between G3 and G4 $F_{(1,10)} = 24.87$; between G3 and G5 $F_{(1,10)} = 24.31$) (2 kHz between G1 and G2 $F_{(1,10)} = 20.98$; between G1 and G4 $F_{(1,10)} = 22.40$; between G1 and G5 $F_{(1,10)} = 21.46$; between G3 and G2 $F_{(1,10)} = 14.99$; between G3 and G4 $F_{(1,10)} = 16.19$; between G3 and G5 $F_{(1,10)} = 15.40$)}. ***This finding indicated that 0.5 and 2 kHz ABR were very sensitive to low frequency band noise, or the combination of low plus high frequency band noises.***

ANOVA analysis showed no main group effects at 4kHz ($F_{(4,10)}$ was 0.25, $p>0.05$). Bonferroni post hoc analysis showed no significant difference in the amplitudes of 4 kHz ABR between the five groups, $p>0.05$ (between G1 and G2 $F_{(1,10)} = 0.45$; between G1 and G3 $F_{(1,10)} = 0.25$; between G1 and G4 $F_{(1,10)} = 0.73$; between G1 and G5 $F_{(1,10)} = 0.70$; between G2 and G3 $F_{(1,10)} = 0.03$; between G2 and G4 $F_{(1,10)} = 0.03$; between G2 and G5 $F_{(1,10)} = 0.03$; between G3 and G4 $F_{(1,10)} = 0.12$; between G3 and G5 $F_{(1,10)} = 0.11$; between G4 and G5 $F_{(1,10)} = 0.00$).

At 6 kHz and 8 kHz, main effects were noted ($F_{(4,10)}$ were 18.34 and 33.72 respectively, $p<0.01$). Bonferroni post hoc analysis showed that in terms of the amplitudes of 6 kHz and 8 kHz ABR, there were no significant differences between group 1 and group 2, $p>0.05$ (6 kHz $F_{(1,10)} = 0.63$; 8 kHz $F_{(1,10)} = 0.56$). In terms of the amplitudes of 6 kHz and 8 kHz ABR, no significant differences were observed between groups 3, 4 and 5, $p>0.05$ {(6 kHz between G3 and G4 $F_{(1,10)} = 0.06$; between G3 and G5 $F_{(1,10)} = 0.12$; between G4 and G5 $F_{(1,10)} = 0.00$) (8 kHz between G3 and G4 $F_{(1,10)} = 0.06$; between G3 and G5 $F_{(1,10)} = 0.09$; between G4 and G5 $F_{(1,10)} = 0.00$)}.

However, in term of the amplitudes of 6 kHz and 8 kHz ABR, both group 1 and group 2 were significantly higher than groups 3, 4, or 5, respectively, $p<0.05$ {(6 kHz between G1 and G3 $F_{(1,10)} = 32.43$; between G1 and G4 $F_{(1,10)} = 35.39$; between G1 and G5 $F_{(1,10)} = 36.54$; between G2 and G3 $F_{(1,10)} = 24.04$; between G2 and G4 $F_{(1,10)} = 26.60$; between G2 and G5 $F_{(1,10)} = 27.60$) (8 kHz between G1 and G3 $F_{(1,10)} = 58.85$; between G1 and G4 $F_{(1,10)} = 62.73$; between G1 and G5 $F_{(1,10)} = 63.47$; between G2 and G3 $F_{(1,10)} = 47.90$; between G2 and G4 $F_{(1,10)} = 51.41$; between G2 and G5 $F_{(1,10)} = 52.08$)}. ***This finding indicated that high frequency band noise, or the combination of high plus low frequency band noises, could specifically reduce the amplitude of 6/8 kHz ABR.***

3.2.3. The Changes in CM as a Function of NIHL

Ten figures were developed to show the amplitudes of CM RW (Figures 3-31 through 3-35) and CM EC (Figures 3-36 through 3-40), respectively.

Table 3-7: The Amplitudes of CM RW in the Five Groups

	Ear No.	1	2	3	4	5	6	Mean	SD
0.5 kHz	Group 1	50.07	49.10	46.74	48.08	49.09	48.39	48.58	1.13
	Group 2*	19.15	19.60	21.63	23.47	20.66	20.77	20.88	1.55
	Group 3	47.89	49.87	44.71	43.66	46.82	45.76	46.45	2.24
	Group 4*	17.35	19.74	20.94	20.16	20.48	20.92	19.93	1.35
	Group 5*	20.91	22.32	19.58	18.32	20.89	20.40	20.40	1.36
2 kHz	Group 1	38.04	39.59	36.34	35.79	37.45	36.89	37.35	1.35
	Group 2*	21.85	22.63	19.69	17.96	21.77	21.03	20.82	1.72
	Group 3	32.69	33.59	34.49	35.40	36.32	39.08	35.26	2.27
	Group 4*	21.02	22.37	19.01	18.21	20.42	19.75	20.13	1.48
	Group 5*	20.40	21.29	18.54	19.48	22.16	21.46	20.55	1.36
4 kHz	Group 1	35.61	35.60	32.12	33.80	35.22	34.89	34.54	1.36
	Group 2	33.19	33.77	30.80	32.24	35.18	33.78	33.16	1.50
	Group 3	34.17	34.74	33.60	31.12	33.78	33.62	33.50	1.24
	Group 4	30.65	31.60	32.54	33.47	34.40	34.23	32.81	1.50
	Group 5	34.30	35.05	31.85	30.62	33.51	32.69	33.00	1.63
6 kHz	Group 1	31.86	30.50	28.71	28.03	29.94	29.35	29.73	1.36
	Group 2	29.06	29.04	24.11	25.61	27.25	27.33	27.06	1.94
	Group 3*	16.10	17.13	15.05	18.16	19.19	21.14	17.80	2.20
	Group 4*	15.50	16.31	17.05	17.71	18.30	19.76	17.44	1.51
	Group 5*	16.77	17.58	15.00	15.91	19.97	18.34	17.26	1.78
8 kHz	Group 1	18.02	19.23	15.84	14.98	17.36	16.63	17.01	1.53
	Group 2	16.59	15.42	13.23	14.30	18.01	17.81	15.89	1.92
	Group 3*	8.48	9.50	10.28	10.87	12.35	11.34	10.47	1.37
	Group 4*	10.83	12.11	8.90	7.87	10.35	9.72	9.96	1.49
	Group 5*	10.64	11.97	8.93	7.75	10.30	9.76	9.89	1.45

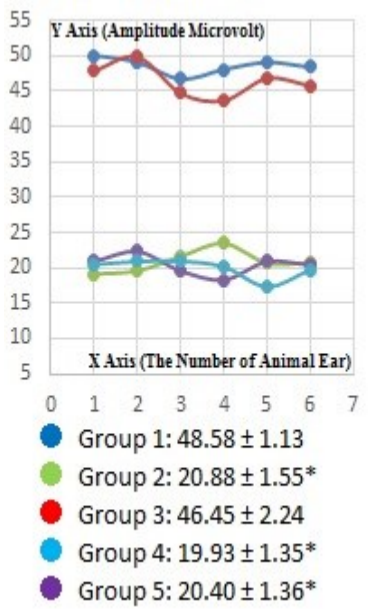
At 0.5 kHz and 2 kHz, * indicates significantly different from group 1 and group 3, $P<.05$; there were no significant differences between group 1 and 3, and among group 2, 4 and 5, $P>.05$. At 4 kHz, there were no significant differences among the five groups, $P>.05$. At 6 kHz and 8 kHz, * indicates significantly different from group 1 and group 2, $P<.05$; there were no significant differences between group 1 and 2, and among group 3, 4 and 5, $P>.05$. The amplitudes of CM RW were in microvolt.

Table 3-8: The Amplitudes of CM EC in the Five Groups

	Ear No.	1	2	3	4	5	6	Mean	SD
0.5 kHz	Group 1	5.46	5.26	4.54	5.08	5.20	5.15	5.11	0.31
	Group 2*	1.75	1.84	2.42	2.56	2.18	2.29	2.17	0.32
	Group 3	5.08	5.25	4.57	4.52	4.79	4.65	4.81	0.29
	Group 4*	1.67	1.78	2.21	2.08	2.31	2.46	2.09	0.31
	Group 5*	2.36	2.48	1.80	1.70	2.24	2.13	2.12	0.31
2 kHz	Group 1	4.23	4.60	3.81	3.70	4.16	4.10	4.10	0.32
	Group 2*	2.11	2.42	1.89	1.73	2.08	1.95	2.03	0.24
	Group 3	3.71	3.79	3.85	3.91	3.98	4.34	3.93	0.22
	Group 4*	2.02	2.32	1.82	1.67	1.99	1.87	1.95	0.22
	Group 5*	1.88	2.03	1.69	1.84	2.36	2.06	1.98	0.23
4 kHz	Group 1	3.94	3.90	3.17	3.38	3.67	3.39	3.58	0.31
	Group 2	3.22	3.65	2.98	3.16	3.87	3.68	3.43	0.35
	Group 3	3.72	3.81	3.25	3.03	3.60	3.25	3.44	0.31
	Group 4	2.85	3.23	3.38	3.53	3.72	3.55	3.38	0.31
	Group 5	3.60	3.78	3.14	2.91	3.57	3.39	3.40	0.32
6 kHz	Group 1	3.66	3.23	2.81	2.62	3.23	3.09	3.11	0.36
	Group 2	3.40	2.99	2.45	2.61	2.81	2.92	2.86	0.33
	Group 3*	1.06	1.08	1.25	1.44	1.64	1.69	1.36	0.27
	Group 4*	1.03	1.06	1.22	1.40	1.65	1.58	1.32	0.26
	Group 5*	1.20	1.37	1.02	1.05	1.61	1.57	1.30	0.26
8 kHz	Group 1	2.25	2.31	2.02	1.36	2.22	2.06	2.04	0.35
	Group 2	1.95	1.94	1.57	1.67	2.12	2.08	1.89	0.22
	Group 3*	0.28	0.56	0.65	0.69	0.96	0.78	0.65	0.23
	Group 4*	0.76	0.94	0.55	0.27	0.68	0.64	0.64	0.22
	Group 5*	0.75	0.92	0.54	0.27	0.66	0.63	0.63	0.22

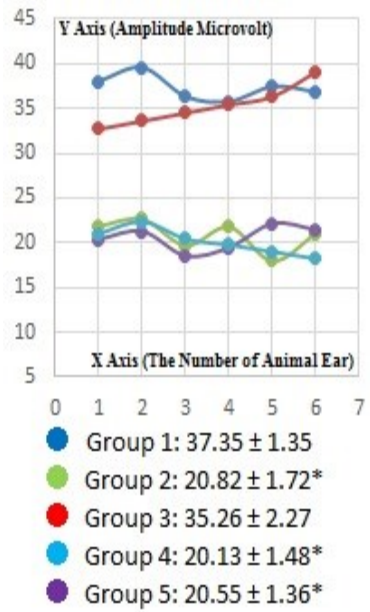
At 0.5 kHz and 2 kHz, * indicates significantly different from group 1 and group 3, $P<.05$; there were no significant differences between group 1 and 3, and among group 2, 4 and 5, $P>.05$. At 4 kHz, there were no significant differences among the five groups, $P>.05$. At 6 kHz and 8 kHz, * indicates significantly different from group 1 and group 2, $P<.05$; there were no significant differences between group 1 and 2, and among group 3, 4 and 5, $P>.05$.

The amplitudes of CM EC were in microvolt.



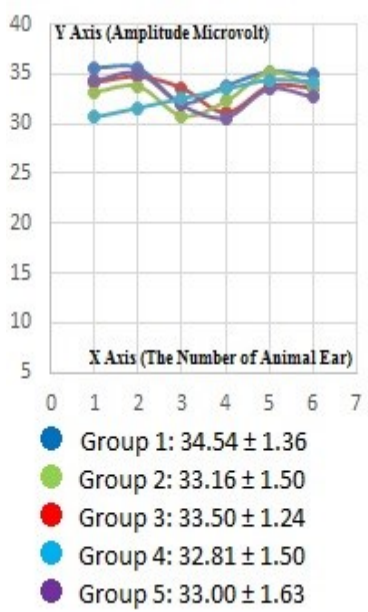
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 264.638$, $df=4$)
* indicates significant reduction in the amplitudes of 0.5 kHz CM RW were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-31. The Amplitudes of 0.5 kHz CM RW in the Five Groups



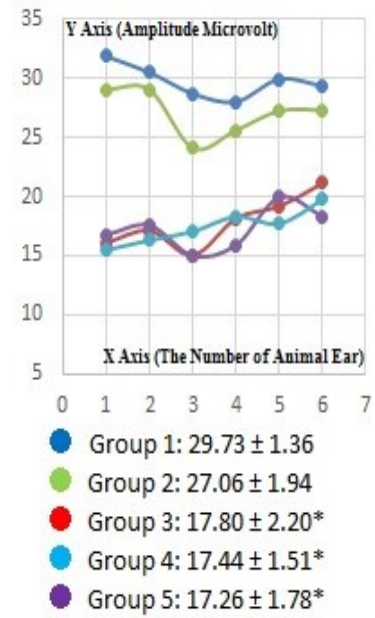
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 84.014$, $df=4$)
* indicates significant reduction in the amplitudes of 2 kHz CM RW were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-32. The Amplitudes of 2 kHz CM RW in the Five Groups



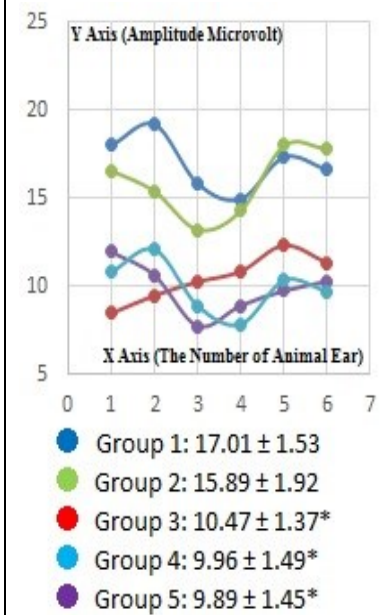
ANOVA showed there were no main group effects between the five groups, ($F_{(4, 10)} = .645$, $df=4$). At 4 kHz, there were no significant differences between the five groups, $p>.05$.

Figure 3-33. The Amplitudes of 4 kHz CM RW in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 34.077$, $df=4$)
* indicates significant reduction in the amplitudes of 6 kHz CM RW were found in group 3, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-34. The Amplitudes of 6 kHz CM RW in the Five Groups

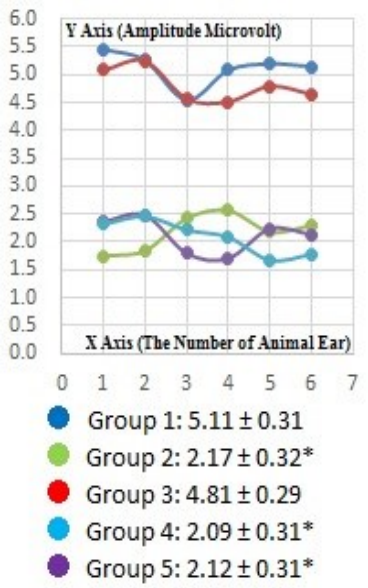


ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 13.610$, $df=4$)
* indicates significant reduction in the amplitudes of 8 kHz CM RW were found in group 3, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-35. The Amplitudes of 8 kHz CM RW in the Five Groups

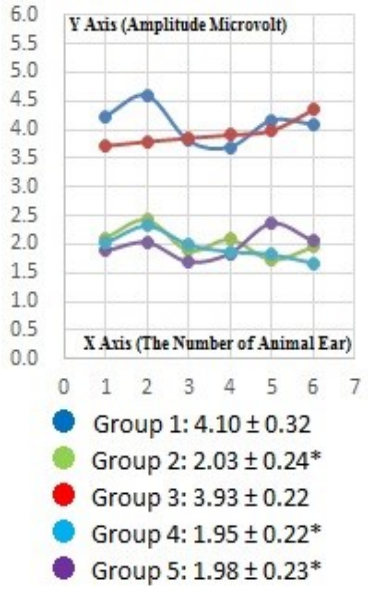
X axis - the number of animal ear, y axis was the amplitude of CM RW in microvolt.
The dark blue curve was is group 1, the green curve was group 2, the red was group 3, the light blue was group 4, and the purple was group 5.

a



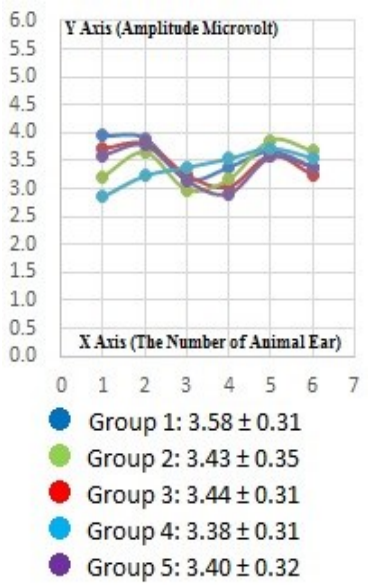
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 68.496$, $df=4$)
* indicates significant reduction in the amplitudes of 0.5 kHz CM EC were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-36. The Amplitudes of 0.5 kHz CM EC in the Five Groups



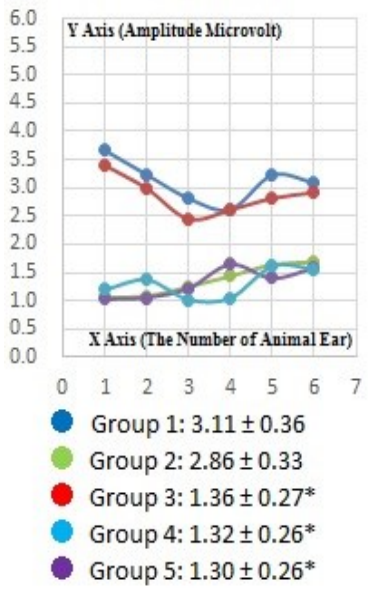
ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 62.610$, $df=4$)
* indicates significant reduction in the amplitudes of 2 kHz CM EC were found in group 2, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 3, and between group 2, 4 and 5, $p>.05$.

Figure 3-37. The Amplitudes of 2 kHz CM EC in the Five Groups



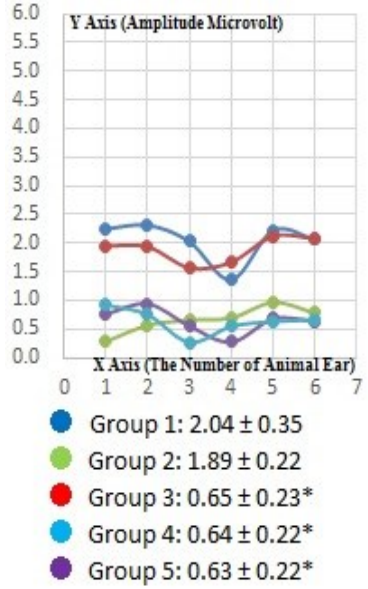
ANOVA showed there were no main group effects between the five groups, ($F_{(4, 10)} = .173$, $df=4$)
At 4 kHz, there were no significant differences between the five groups, $p>.05$.

Figure 3-38. The Amplitudes of 4 kHz CM EC in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 26.506$, $df=4$)
* indicates significant reduction in the amplitudes of 6 kHz CM EC were found in group 3, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-39. The Amplitudes of 6 kHz CM EC in the Five Groups



ANOVA showed there were main group effects between the five groups, ($F_{(4, 10)} = 26.16$, $df=4$)
* indicates significant reduction in the amplitudes of 6 kHz CM EC were found in group 3, 4 and 5, as compared to group 1, $p<.05$; there were no significant differences between group 1 and 2, and between group 3, 4 and 5, $p>.05$.

Figure 3-40. The Amplitudes of 8 kHz CM EC in the Five Groups

X axis - the number of animal ear, y axis was the amplitude of CM EC in microvolt.
The dark blue curve was is group 1, the green curve was group 2, the red was group 3, the light blue was group 4, and the purple was group 5.

There were changes in the amplitudes of CM between the five groups. The highest amplitude of CM was seen in the normal group, and the amplitude of CM was reduced after noise exposure. The changes in amplitudes of CM between the five groups were analyzed using repeated measures ANOVA.

Analysis showed main group effects at 0.5 kHz and 2 kHz (CM RW $F_{(4,10)}$ were 264.64 and 84.01 respectively, $p<0.05$; 0.5 kHz and 2 kHz CM EC $F_{(4,10)}$ were 68.50 and 62.61 respectively, $p<0.05$). To examine specific group differences, Bonferroni post hoc analysis were conducted. In terms of the amplitudes of 0.5 kHz and 2 kHz ABR, there were no significant differences between groups 1 and group 3, $p>0.05$ {(0.5 kHz CM RW $F_{(1,10)} = 2.70$) (0.5 kHz CM EC $F_{(1,10)} = 1.31$) (2 kHz CM RW $F_{(1,10)} = 2.42$) (2 kHz CM EC $F_{(1,10)} = 0.72$)}.

In terms of the amplitudes of 0.5 and 2 kHz CM, there was no significant difference between groups 2, 4 and 5, $p>0.05$ {(0.5 kHz CM RW between G2 and G4 $F_{(1,10)} = 0.54$; between G2 and G5 $F_{(1,10)} = 0.13$; between G4 and G5 $F_{(1,10)} = 0.13$) (0.5 kHz CM EC between G2 and G4 $F_{(1,10)} = 0.11$; between G2 and G5 $F_{(1,10)} = 0.05$; between G4 and G5 $F_{(1,10)} = 0.01$) (2 kHz CM RW between G2 and G4 $F_{(1,10)} = 0.27$; between G2 and G5 $F_{(1,10)} = 0.04$; between G4 and G5 $F_{(1,10)} = 0.10$) (2 kHz CM EC between G2 and G4 $F_{(1,10)} = 0.17$; between G2 and G5 $F_{(1,10)} = 0.07$; between G4 and G5 $F_{(1,10)} = 0.02$)}.

The amplitudes of 0.5 kHz and 2 kHz CM in both group 1 and group 3 were significantly higher than those in groups 2, 4, or 5 respectively, $p<0.05$ {(0.5 kHz CM RW between G1 and G2 $F_{(1,10)} = 459.01$; between G1 and G4 $F_{(1,10)} = 491.00$; between G1 and G5 $F_{(1,10)} = 474.87$; between G3 and G2 $F_{(1,10)} = 391.25$; between G3 and G4 $F_{(1,10)} = 420.83$; between G3 and G5 $F_{(1,10)} =$

405.91) (0.5 kHz CM EC between G1 and G2 $F_{(1,10)}$ = 122.03; between G1 and G4 $F_{(1,10)}$ = 129.55; between G1 and G5 $F_{(1,10)}$ = 126.78; between G3 and G2 $F_{(1,10)}$ = 98.04; between G3 and G4 $F_{(1,10)}$ = 104.78; between G3 and G5 $F_{(1,10)}$ = 102.31) (2 kHz CM RW between G1 and G2 $F_{(1,10)}$ = 151.88; between G1 and G4 $F_{(1,10)}$ = 164.91; between G1 and G5 $F_{(1,10)}$ = 156.89; between G3 and G2 $F_{(1,10)}$ = 115.95; between G3 and G4 $F_{(1,10)}$ = 127.36; between G3 and G5 $F_{(1,10)}$ =120.32) (2 kHz EC between G1 and G2 $F_{(1,10)}$ = 108.06; between G1 and G4 $F_{(1,10)}$ = 116.85; between G1 and G5 $F_{(1,10)}$ = 113.52; between G3 and G2 $F_{(1,10)}$ = 91.19; between G3 and G4 $F_{(1,10)}$ = 99.27; between G3 and G5 $F_{(1,10)}$ = 96.20)}. ***This finding meant that low frequency band noise, or the combination of low plus high frequency band noises, could specifically cause reduction in the amplitudes of 0.5/2 kHz CM.***

At 4 kHz, no main group effects were noted (CM RW and CM EC $F_{(4,10)}$ were 0.645 and 0.037 respectively, $p>0.05$). Bonferroni post hoc analysis showed no significant difference in the amplitudes of 4 kHz ABR between the five groups, $p>0.05$ {(CM RW between G1 and G2 $F_{(1,10)}$ = 1.32; between G1 and G3 $F_{(1,10)}$ = 0.74; between G1 and G4 $F_{(1,10)}$ = 2.06; between G1 and G5 $F_{(1,10)}$ = 1.63; between G2 and G3 $F_{(1,10)}$ = 0.08; between G2 and G4 $F_{(1,10)}$ = 0.08; between G2 and G5 $F_{(1,10)}$ = 0.02; between G3 and G4 $F_{(1,10)}$ = 0.33; between G3 and G5 $F_{(1,10)}$ = 0.17; between G4 and G5 $F_{(1,10)}$ = 0.02) (CM EC between G1 and G2 $F_{(1,10)}$ = 0.31; between G1 and G3 $F_{(1,10)}$ = 0.25; between G1 and G4 $F_{(1,10)}$ = 0.56; between G1 and G5 $F_{(1,10)}$ = 0.45; between G2 and G3 $F_{(1,10)}$ = 0.00; between G2 and G4 $F_{(1,10)}$ = 0.04; between G2 and G5 $F_{(1,10)}$ = 0.01; between G3 and G4 $F_{(1,10)}$ = 0.06; between G3 and G5 $F_{(1,10)}$ = 0.03; between G4 and G5 $F_{(1,10)}$ = 0.01}.

At 6 kHz (CM RW and CM EC - $F_{(4,10)}$ = 34.08 and 26.51 respectively) and 8 kHz (CM RW and CM EC - $F_{(4,10)}$ =13.61 and 26.16 respectively $p<0.01$), indicated main group effects. Bonferroni post hoc analysis showed, that in terms of the amplitudes of 6 kHz and 8 kHz CM, there were no significant differences between group 1 and group 2, $p>0.05$ {(6 kHz CM RW $F_{(1,10)}$ = 3.31) (6 kHz CM EC $F_{(1,10)}$ = 0.96) (8 kHz CM RW $F_{(1,10)}$= 0.69) (8 kHz CM EC $F_{(1,10)}$= 0.56)}

In terms of the amplitudes of 6 kHz and 8 kHz CM, no significant difference was observed between groups 3, 4 and 5, $p>0.05$ {(6 kHz CM RW between G3 and G4 $F_{(1,10)}$ = 0.06; between G3 and G5 $F_{(1,10)}$ = 0.13; between G4 and G5 $F_{(1,10)}$ = 0.01) (6 kHz CM EC between G3 and G4 $F_{(1,10)}$ =0.02; between G3 and G5 $F_{(1,10)}$ = 0.05; between G4 and G5 $F_{(1,10)}$ = 0.01) (8 kHz CM RW between G3 and G4 $F_{(1,10)}$ = 0.14; between G3 and G5 $F_{(1,10)}$ = 0.18; between G4 and G5 $F_{(1,10)}$ = 0.00) (8 kHz CM EC between G3 and G4 $F_{(1,10)}$ = 0.01; between G3 and G5 $F_{(1,10)}$ = 0.02; between G4 and G5 $F_{(1,10)}$ = 0.00)}

However, in terms of the amplitudes of 6 kHz and 8 kHz CM, both group 1 and group 2 was significantly higher than groups 3, 4, or 5 respectively, $p<0.05$ {(6 kHz CM RW between G1 and G3 $F_{(1,10)}$ = 66.39; between G1 and G4 $F_{(1,10)}$ = 70.41; between G1 and G5 $F_{(1,10)}$ = 72.45; between G2 and G3 $F_{(1,10)}$ = 40.04; between G2 and G4 $F_{(1,10)}$ = 43.18; between G2 and G5 $F_{(1,10)}$ = 44.77) (6 kHz CM EC between G1 and G3 $F_{(1,10)}$ = 48.70; between G1 and G4 $F_{(1,10)}$ = 50.76; between G1 and G5 $F_{(1,10)}$ = 51.95; between G2 and G3 $F_{(1,10)}$ = 36.01; between G2 and G4 $F_{(1,10)}$ = 37.79; between G2 and G5 $F_{(1,10)}$ = 38.81) (8 kHz CM RW between G1 and G3 $F_{(1,10)}$ = 23.72; between G1 and G4 $F_{(1,10)}$ =27.52; between G1 and G5 $F_{(1,10)}$ = 28.08; between G2 and G3 $F_{(1,10)}$ =16.31; between G2 and G4 $F_{(1,10)}$ = 19.49; between G2 and G5 $F_{(1,10)}$ = 19.96) (8 kHz CM EC between

G1 and G3 $F_{(1,10)} = 47.49$; between G1 and G4 $F_{(1,10)} = 48.57$; between G1 and G5 $F_{(1,10)} = 49.27$; between G2 and G3 $F_{(1,10)} = 37.70$; between G2 and G4 $F_{(1,10)} = 38.67$; between G2 and G5 $F_{(1,10)} = 39.29$)}. ***This finding indicated that the amplitudes of 6/8 kHz CM were sensitive to the high frequency band noise, or high plus low frequency band noises.***

3.3. Research Question 3 - What is The Relationship between the CM Measured at the RW and the CM Measured at the EC in Normal Hearing and NIHL?

In each animal ear, CM were recorded at two locations, round window (CM RW) and ear canal (CM EC). There were five groups in the research, in each group the correlation between CM RW and CM EC was investigated respectively. In order to determine the statistical relationship, or association, between two continuous variables, Pearson's correlation coefficient is the statistical option. In the current study, Pearson correlation coefficients were used to analyze the relationship between the CM RW) and the CM EC. Correlations were considered significant when $p < 0.05$.

CM RW and ear CM EC were recorded in the five groups. In each group, both the CM RW and CM EC were measured at five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. All amplitudes of CM RW and CM EC in group 1 are presented in 3-9; the amplitudes of CM RW and CM EC in group 2 were presented in Table 3-10, the amplitudes of CM RW and CM EC in group 3 were presented in Table 3-11, the amplitudes of CM RW and CM EC in group 4 were presented in Table 3-12, and the amplitudes of CM RW and CM EC in group 5 were presented in Table 3-13. The unit for the amplitude of CM was microvolt.

In each animal ear, CM RW and CM EC were recorded one after another. In the recording of CM RW and CM EC, the evoking stimulus and response generators were the same, both the CM

RW and CM EC were generated by the outer hair cells and inner hair cells, but mainly by the outer hair cells. The difference between the recording of CM RW and CM EC were the locations of primary electrode, in the recording of CM RW, the primary electrode was placed on the round window; in the recording of CM EC, the primary electrode was placed in the ear canal, which was a non-invasive method.

There were five groups in the research, and CMs were recorded at five frequencies. In each group there were three animals, six ears that were denoted by the Arabic numbers from 1 to 6. So in each group, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively.

Table 3-9: The Correlation between CM RW and CM EC in Group 1

The Amplitudes of Cochlear Microphonic in Group 1									
	Ear No.	1	2	3	4	5	6	PCC	*P* value
0.5 kHz	CM RW	50.07	49.10	46.74	48.08	49.09	48.39	0.968	*P<0.05*
	CM EC	5.46	5.26	4.54	5.08	5.20	5.15		
	RW/EC	9.18	9.34	10.29	9.46	9.45	9.40		
2 kHz	CM RW	38.04	39.59	36.34	35.79	37.45	36.89	0.982	*P<0.05*
	CM EC	4.23	4.60	3.81	3.70	4.16	4.10		
	RW/EC	8.98	8.61	9.55	9.68	9.00	9.01		
4 kHz	CM RW	35.61	35.60	32.12	33.80	35.22	34.89	0.876	*P<0.05*
	CM EC	3.94	3.90	3.17	3.38	3.67	3.39		
	RW/EC	9.03	9.12	10.13	10.00	9.60	10.29		
6 kHz	CM RW	31.86	30.50	28.71	28.03	29.94	29.35	0.986	*P<0.05*
	CM EC	3.66	3.23	2.81	2.62	3.23	3.09		
	RW/EC	8.70	9.43	10.20	10.70	9.28	9.50		
8 kHz	CM RW	18.02	19.23	15.84	14.98	17.36	16.63	0.846	*P<0.05*
	CM EC	2.25	2.31	2.02	1.36	2.22	2.06		
	RW/EC	8.03	8.32	7.83	11.05	7.82	8.06		

In group 1, both the CM RW and CM EC were measured in each ear at the five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. There were 3 animals, six ears in group 1 indicated by the Arabic numbers from 1 to 6. Thus, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively. In group 1, there was a strong correlation between CM RW and CM EC, $p<0.05$. Pearson Correlation Coefficients (PCC) were calculated.

Table 3-10: The Correlation between CM RW and CM EC in Group 2

The Amplitudes of Cochlear Microphonic in Group 2									
	Ear No.	1	2	3	4	5	6	PCC	*P* value
0.5 kHz	CM RW	19.15	19.60	21.63	23.47	20.66	20.77	0.94	*P<0.05*
	CM EC	1.75	1.84	2.42	2.56	2.18	2.29		
	RW/EC	10.94	10.65	8.94	9.16	9.47	9.05		
2 kHz	CM RW	21.85	22.63	19.69	17.96	21.77	21.03	0.909	*P<0.05*
	CM EC	2.11	2.42	1.89	1.73	2.08	1.95		
	RW/EC	10.37	9.34	10.44	10.40	10.46	10.79		
4 kHz	CM RW	33.19	33.77	30.80	32.24	35.18	33.78	0.935	*P<0.05*
	CM EC	3.22	3.65	2.98	3.16	3.87	3.68		
	RW/EC	10.32	9.25	10.33	10.19	9.09	9.18		
6 kHz	CM RW	29.06	29.04	24.11	25.61	27.25	27.33	0.911	*P<0.05*
	CM EC	3.40	2.99	2.45	2.61	2.81	2.92		
	RW/EC	8.55	9.71	9.85	9.81	9.68	9.37		
8 kHz	CM RW	16.59	15.42	13.23	14.30	18.01	17.81	0.973	*P<0.05*
	CM EC	1.95	1.94	1.57	1.67	2.12	2.08		
	RW/EC	8.50	7.97	8.45	8.59	8.50	8.56		

In group 2, both the CM RW and CM EC were measured in each ear at the five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. There were 3 animals, six ears in group 2, indicated by the Arabic numbers from 1 to 6. Thus, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively. In group 2, there was a strong correlation between the CM RW and CM EC, $p<0.05$.

Table 3-11: The Correlation between CM RW and CM EC in Group 3

The Amplitudes of Cochlear Microphonic in Group 3									
	Ear No.	1	2	3	4	5	6	PCC	*P* value
0.5 kHz	CM RW	47.89	49.87	44.71	43.66	46.82	45.76	0.976	*P<0.05*
	CM EC	5.08	5.25	4.57	4.52	4.79	4.65		
	RW/EC	9.43	9.51	9.79	9.67	9.77	9.84		
2 kHz	CM RW	32.69	33.59	34.49	35.40	36.32	39.08	0.984	*P<0.05*
	CM EC	3.71	3.79	3.85	3.91	3.98	4.34		
	RW/EC	8.80	8.86	8.96	9.06	9.13	9.00		
4 kHz	CM RW	34.17	34.74	33.60	31.12	33.78	33.62	0.841	*P<0.05*
	CM EC	3.72	3.81	3.25	3.03	3.60	3.25		
	RW/EC	9.19	9.13	10.35	10.27	9.39	10.34		
6 kHz	CM RW	16.10	17.13	15.05	18.16	19.19	21.14	0.842	*P<0.05*
	CM EC	1.06	1.08	1.25	1.44	1.64	1.69		
	RW/EC	15.13	15.81	12.05	12.66	11.72	12.52		
8 kHz	CM RW	8.48	9.50	10.28	10.87	12.35	11.34	0.982	*P<0.05*
	CM EC	0.28	0.56	0.65	0.69	0.96	0.78		
	RW/EC	30.57	16.84	15.75	15.71	12.80	14.58		

In group 3, both the CM RW and CM EC were measured in each ear at the five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. There were 3 animals, six ears in group 3, indicated by the Arabic numbers from 1 to 6. Thus, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively. In group 3, there was a strong correlation between the CM RW and CM EC, $p<0.05$.

Table 3-12: The Correlation between CM RW and CM EC in Group 4

The Amplitudes of Cochlear Microphonic in Group 4									
	Ear No.	1	2	3	4	5	6	PCC	*P* value
0.5 kHz	CM RW	17.35	19.74	20.94	20.16	20.48	20.92	0.844	*P<0.05*
	CM EC	1.67	1.78	2.21	2.08	2.31	2.46		
	RW/EC	10.38	11.12	9.46	9.68	8.84	8.52		
2 kHz	CM RW	21.02	22.37	19.01	18.21	20.42	19.75	0.985	*P<0.05*
	CM EC	2.02	2.32	1.82	1.67	1.99	1.87		
	RW/EC	10.42	9.65	10.46	10.92	10.25	10.57		
4 kHz	CM RW	30.65	31.60	32.54	33.47	34.40	34.23	0.963	*P<0.05*
	CM EC	2.85	3.23	3.38	3.53	3.72	3.55		
	RW/EC	10.74	9.79	9.63	9.48	9.25	9.64		
6 kHz	CM RW	15.50	16.31	17.05	17.71	18.30	19.76	0.915	*P<0.05*
	CM EC	1.03	1.06	1.22	1.40	1.65	1.58		
	RW/EC	15.04	15.44	13.95	12.66	11.12	12.47		
8 kHz	CM RW	10.83	12.11	8.90	7.87	10.35	9.72	0.98	*P<0.05*
	CM EC	0.76	0.94	0.55	0.27	0.68	0.64		
	RW/EC	14.26	12.88	16.17	29.08	15.30	15.26		

In group 4, both the CM RW and CM EC were measured in each ear at the five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. There were 3 animals, six ears in group 4, indicated by the Arabic numbers from 1 to 6. Thus, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively. In group 4, there was a very strong correlation between the CM RW and CM EC, $p<0.05$.

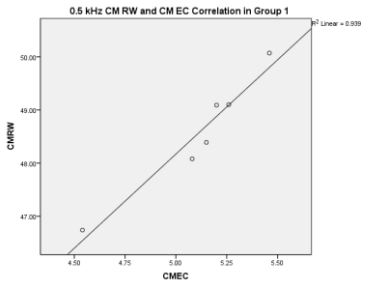
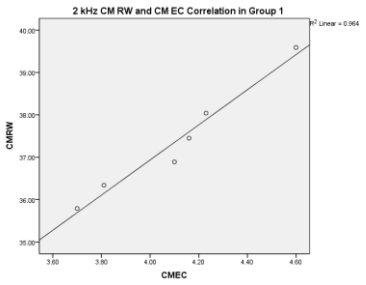
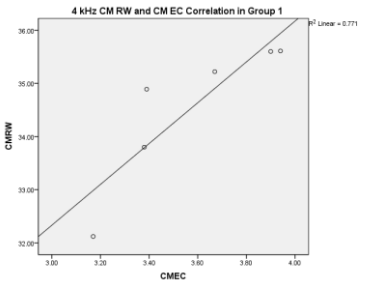
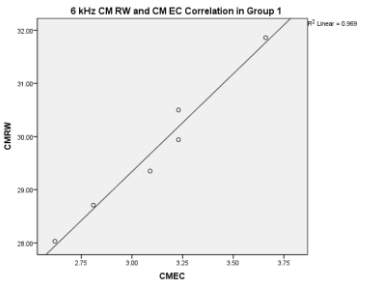
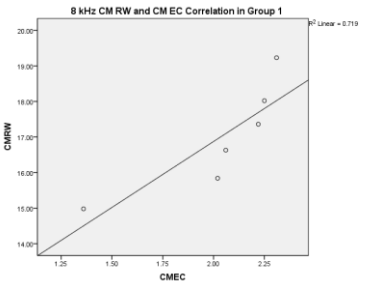
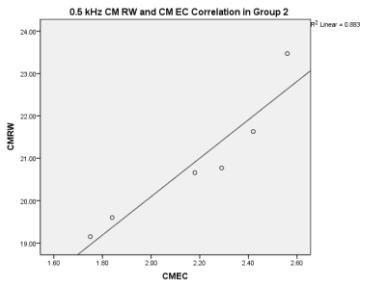
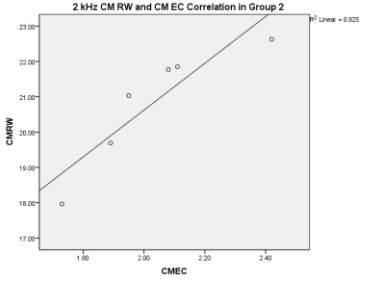
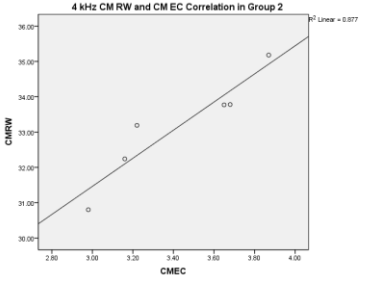
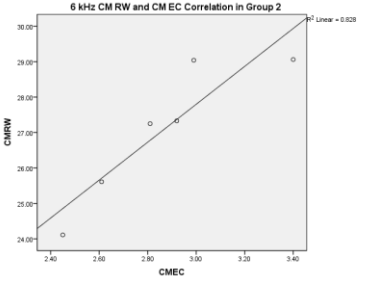
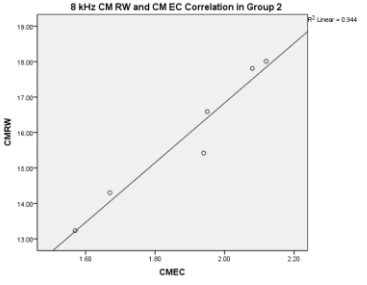
Table 3-13: The Correlation between CM RW and CM EC in Group 5

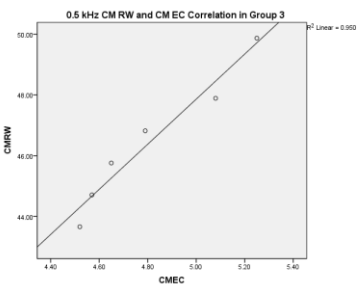
The Amplitudes of Cochlear Microphonic in Group 5									
	Ear No.	1	2	3	4	5	6	PCC	*P* value
0.5 kHz	CM RW	20.91236	22.32119	19.57871	18.31859	20.89425	20.40475	0.954	*P<0.05*
	CM EC	2.360677	2.480538	1.79552	1.703953	2.236554	2.132045		
	RW/EC	8.858631	8.998528	10.9042	10.75064	9.342162	9.570506		
2 kHz	CM RW	20.39606	21.29109	18.5361	19.47713	22.1605	21.4616	0.938	*P<0.05*
	CM EC	1.882362	2.031409	1.691039	1.839914	2.36361	2.058305		
	RW/EC	10.83536	10.48095	10.96136	10.58589	9.375701	10.42683		
4 kHz	CM RW	34.29763	35.05414	31.84519	30.62036	33.50931	32.69118	0.988	*P<0.05*
	CM EC	3.599316	3.775743	3.142656	2.907117	3.565979	3.390326		
	RW/EC	9.52893	9.284036	10.13321	10.5329	9.396947	9.64249		
6 kHz	CM RW	16.77277	17.58391	15.00339	15.91184	19.9696	18.34382	0.961	*P<0.05*
	CM EC	1.195066	1.373027	1.018195	1.048777	1.611818	1.56701		
	RW/EC	14.03501	12.80668	14.73528	15.17181	12.38948	11.70625		
8 kHz	CM RW	10.64026	11.97133	8.928438	7.752101	10.29942	9.763756	0.985	*P<0.05*
	CM EC	0.75041	0.920578	0.544633	0.267714	0.660182	0.630399		
	RW/EC	14.17927	13.00415	16.39349	28.95665	15.60087	15.4882		

In group 5, both the CM RW and CM EC were measured in each ear at the five frequencies, 0.5 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. There were 3 animals, six ears in group 5, indicated by the Arabic numbers from 1 to 6. Thus, at each of the measured frequencies, there were six measurements of CM RW and CM EC respectively. In group 5, there was a very strong correlation between the CM RW and CM EC, $p<0.05$.

In each of the five groups, at each of the measured frequencies, there were strong correlations between the CM RW and CM EC, $p<0.05$; the ratios between CM RW and CM EC (CM RW/CM EC) were also calculated, with most ratios at approximately 9. ***Thus, the CM EC correlates significantly with the CM RW in the normal hearing animals and in the animals with NIHL.***

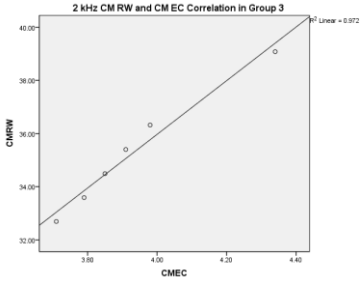
To further illustrate the correlation between CM RW and CM EC well, in each group, at each of the measured frequencies, scatterplot was created and SPSS could yield a regression line, the slope of the regression line corresponds to correlation. R-squared is a statistical value indicating how close the data are to the fitted regression line, and R-squared = PCC^2.

				
In group 1 the Pearson Correlation Coefficients between 0.5 kHz CM RW and 0.5 kHz CM EC was 0.968, *p<0.05*.	In group 1 the Pearson Correlation Coefficients between 2 kHz CM RW and 2 kHz CM EC was 0.982, *p<0.05*.	In group 1 the Pearson Correlation Coefficients between 4 kHz CM RW and 4 kHz CM EC was 0.876, *p<0.05*.	In group 1 the Pearson Correlation Coefficients between 6 kHz CM RW and 6 kHz CM EC was 0.986, *p<0.05*.	In group 1 the Pearson Correlation Coefficients between 8 kHz CM RW and 8 kHz CM EC was 0.846, *p<0.05*.
Figure 3-41. The Correlation between 0.5 kHz CM RW and 0.5 kHz CM EC in Group 1	**Figure 3-42. The Correlation between 2 kHz CM RW and 2 kHz CM EC in Group 1**	**Figure 3-43. The Correlation between 4 kHz CM RW and 4 kHz CM EC in Group 1**	**Figure 3-44. The Correlation between 6 kHz CM RW and 6 kHz CM EC in Group 1**	**Figure 3-45. The Correlation between 8 kHz CM RW and 8 kHz CM EC in Group 1**
				
In group 2 the Pearson Correlation Coefficients between 0.5 kHz CM RW and 0.5 kHz CM EC was 0.94, *p<0.05*.	In group 2 the Pearson Correlation Coefficients between 2 kHz CM RW and 2 kHz CM EC was 0.94, *p<0.05*.	In group 2 the Pearson Correlation Coefficients between 4 kHz CM RW and 4 kHz CM EC was 0.935, *p<0.05*.	In group 2 the Pearson Correlation Coefficients between 6 kHz CM RW and 6 kHz CM EC was 0.911, *p<0.05*.	In group 2 the Pearson Correlation Coefficients between 8 kHz CM RW and 8 kHz CM EC was 0.973, *p<0.05*.
Figure 3-46. The Correlation between 0.5 kHz CM RW and 0.5 kHz CM EC in Group 2	**Figure 3-47. The Correlation between 2 kHz CM RW and 2 kHz CM EC in Group 2**	**Figure 3-48. The Correlation between 4 kHz CM RW and 4 kHz CM EC in Group 2**	**Figure 3-49. The Correlation between 6 kHz CM RW and 6 kHz CM EC in Group 2**	**Figure 3-50. The Correlation between 8 kHz CM RW and 8 kHz CM EC in Group 2**



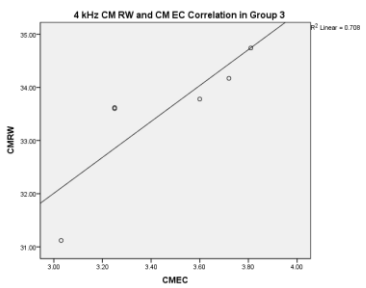
In group 3 the Pearson Correlation Coefficients between 0.5 kHz CM RW and 0.5 kHz CM EC was 0.976, *p<0.05*.

Figure 3-51. The Correlation between 0.5 kHz CM RW and 0.5 kHz CM EC in Group 3



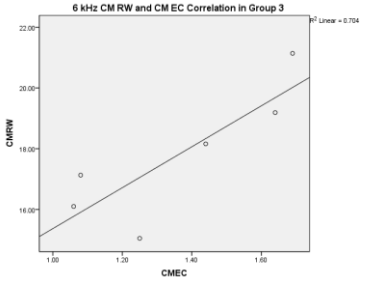
In group 3 the Pearson Correlation Coefficients between 2 kHz CM RW and 2 kHz CM EC was 0.984, *p<0.05*.

Figure 3-52. The Correlation between 2 kHz CM RW and 2 kHz CM EC in Group 3



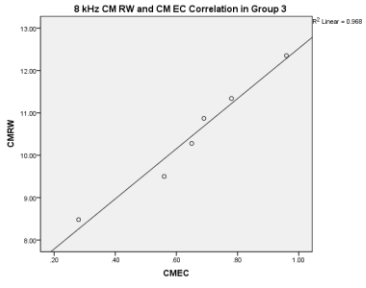
In group 3 the Pearson Correlation Coefficients between 4 kHz CM RW and 4 kHz CM EC was 0.841, *p<0.05*.

Figure 3-53. The Correlation between 4 kHz CM RW and 4 kHz CM EC in Group 3



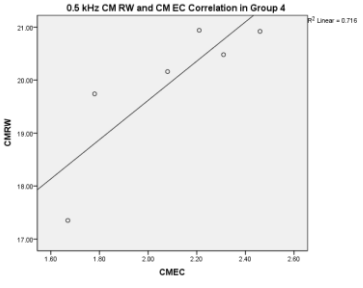
In group 3 the Pearson Correlation Coefficients between 6 kHz CM RW and 6 kHz CM EC was 0.842, *p<0.05*.

Figure 3-54. The Correlation between 6 kHz CM RW and 6 kHz CM EC in Group 3



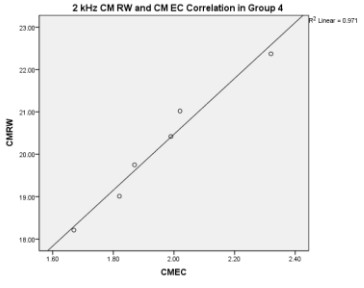
In group 3 the Pearson Correlation Coefficients between 8 kHz CM RW and 8 kHz CM EC was 0.982, *p<0.05*.

Figure 3-55. The Correlation between 8 kHz CM RW and 8 kHz CM EC in Group 3



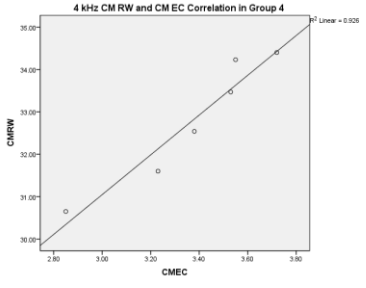
In group 4 the Pearson Correlation Coefficients between 0.5 kHz CM RW and 0.5 kHz CM EC was 0.844, *p<0.05*.

Figure 3-56. The Correlation between 0.5 kHz CM RW and 0.5 kHz CM EC in Group 4



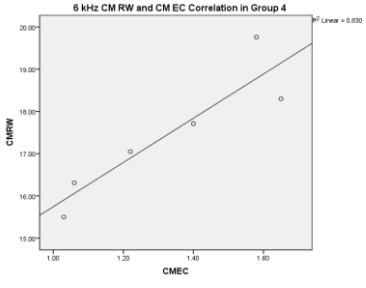
In group 4 the Pearson Correlation Coefficients between 2 kHz CM RW and 2 kHz CM EC was 0.985, *p<0.05*.

Figure 3-57. The Correlation between 2 kHz CM RW and 2 kHz CM EC in Group 4



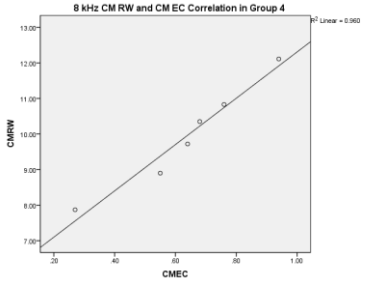
In group 4 the Pearson Correlation Coefficients between 4 kHz CM RW and 4 kHz CM EC was 0.963, *p<0.05*.

Figure 3-58. The Correlation between 4 kHz CM RW and 4 kHz CM EC in Group 4



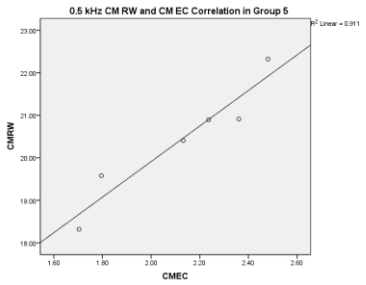
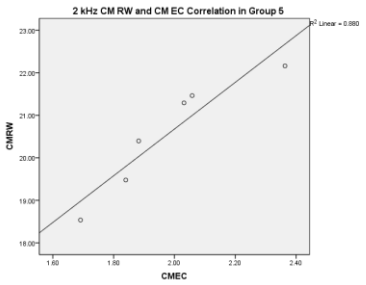
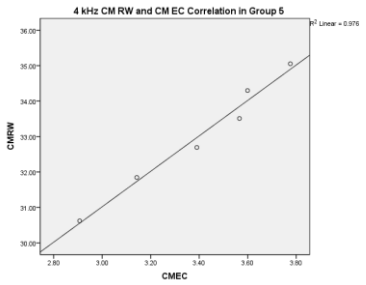
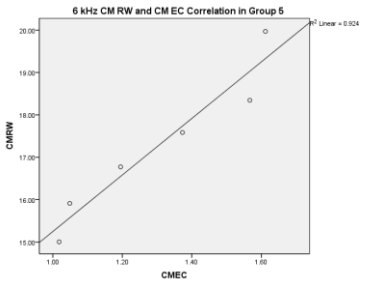
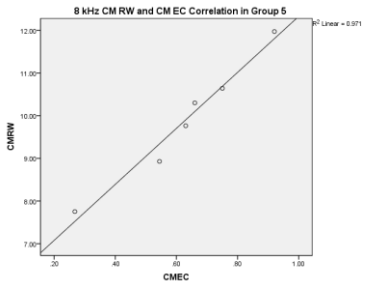
In group 4 the Pearson Correlation Coefficients between 6 kHz CM RW and 6 kHz CM EC was 0.915, *p<0.05*.

Figure 3-59. The Correlation between 6 kHz CM RW and 6 kHz CM EC in Group 4



In group 4 the Pearson Correlation Coefficients between 8 kHz CM RW and 8 kHz CM EC was 0.98, *p<0.05*.

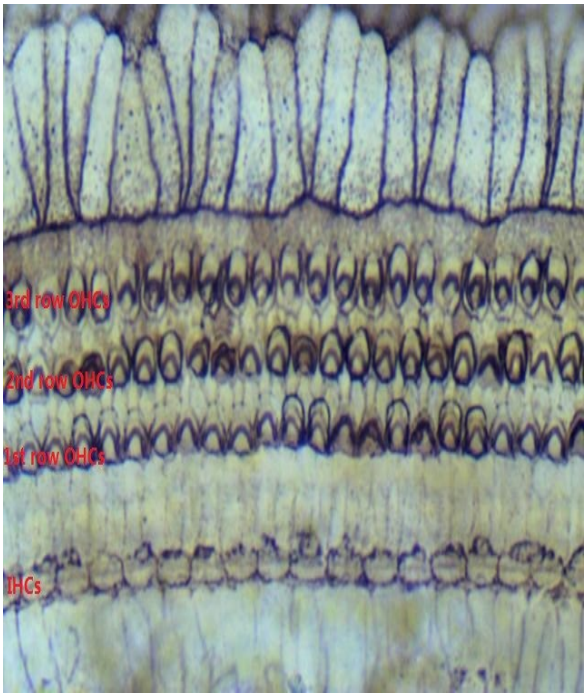
Figure 3-60. The Correlation between 8 kHz CM RW and 8 kHz CM EC in Group 4

				
In group 5 the Pearson Correlation Coefficients between 0.5 kHz CM RW and 0.5 kHz CM EC was 0.954, *p<0.05*.	In group 5 the Pearson Correlation Coefficients between 2 kHz CM RW and 2 kHz CM EC was 0.938, *p<0.05*.	In group 5 the Pearson Correlation Coefficients between 4 kHz CM RW and 4 kHz CM EC was 0.988, *p<0.05*.	In group 5 the Pearson Correlation Coefficients between 6 kHz CM RW and 6 kHz CM EC was 0.961, *p<0.05*.	In group 5 the Pearson Correlation Coefficients between 8 kHz CM RW and 8 kHz CM EC was 0.985, *p<0.05*.
Figure 3-61. The Correlation between 0.5 kHz CM RW and 0.5 kHz CM EC in Group 5	**Figure 3-62. The Correlation between 2 kHz CM RW and 2 kHz CM EC in Group 5**	**Figure 3-63. The Correlation between 4 kHz CM RW and 4 kHz CM EC in Group 5**	**Figure 3-64. The Correlation between 6 kHz CM RW and 6 kHz CM EC in Group 5**	**Figure 3-65. The Correlation between 8 kHz CM RW and 8 kHz CM EC in Group 5**

3.4 Research Question 4 – What is the Difference in the Morphology of and Number of Missing OHCs as a result of NIHL?

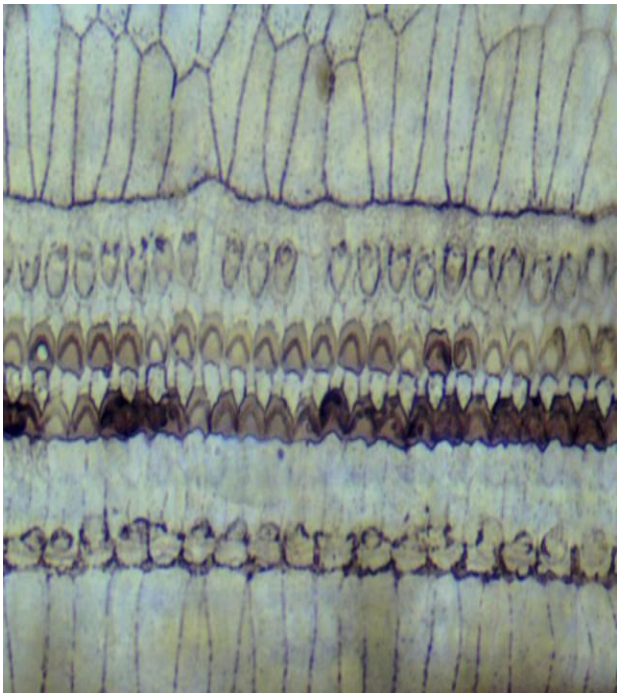
3.4.1. The Morphological Study of the Organ of Corti

In group 1 (normal control) most of the OHCs were intact and less change was observed (Figure 3-66). In the noise exposure groups, mild morphological changes were noted (i.e., swelling), but only a few OHCs were missing (Figure 3-67).



This specimen was taken close to cochlear apex from the normal animal. The outer hair cells were intact and almost no change was observed.

Figure 3-66. The Normal Outer Hair Cells



The specimen was taken close to cochlear apex from group 4 which was exposed to low then high frequency band-noises. Compared to the normal outer hair cells, some outer hair cells in this specimen were slightly swollen, but only a few outer hair cells were missing.

Figure 3-67. The Outer Hair Cells after Noise Exposure

3.4.2. Comparing the Numbers of Missing OHCs between the Five Groups

The full basilar membrane was removed from each animal. In the normal group, the numbers of the missing OHCs from the full basilar membrane were counted, and this number represented the normal control.

In the noise exposure groups, the numbers of the missing OHCs from the apex region was considered to be a result of low frequency band-noises exposure; the number of the missing outer hair cells from the basal turn was considered to be a result of high frequency band-noises exposure. However, in the noise exposure groups, the numbers of the missing OHCs from the full basilar membrane were the final numbers used for comparison.

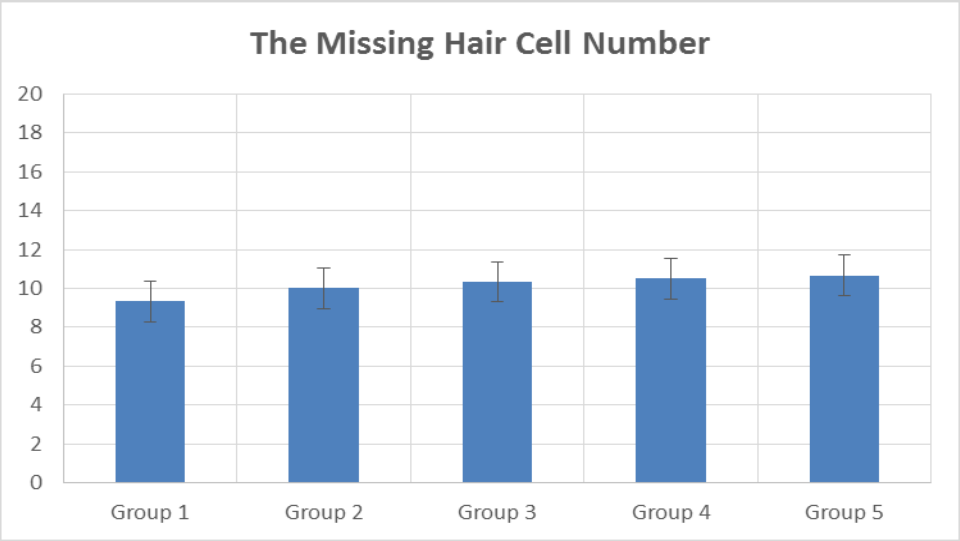
The numbers of the missing OHCs are presented in Table 3-14. In each group, there were three animals, six ears which were indicated by Arabic numbers from 1 to 6. The data in column 1 and 2 were the numbers from one animal's left and right ear respectively. The data in column 3 and 4 were the numbers from one animal's left and right ear respectively. The data in column 5 and 6 were the numbers from one animal's left and right ear respectively.

Table 3-14. The Numbers of the Missing Outer Hair Cell in the Five Groups

	1	2	3	4	5	6	Mean	SD
Group 1	10	8	9	9	11	9	9.333333	1.032796
Group 2	11	11	10	10	9	9	10	0.894427
Group 3	12	11	11	9	9	10	10.33333	1.21106
Group 4	11	11	12	10	10	9	10.5	1.048809
Group 5	12	11	11	9	10	11	10.66667	1.032796

In the first row, the Arabic number from 1-6 indicated the number of animal ear.

ANOVA analysis showed no main group effects ($F_{(4,10)} = 1.111$, $p>0.05$). Bonferroni post hoc analysis showed no significant difference between the five groups for the numbers of missing hair cells, $p>0.05$ (between G1 and G2 $F_{(1,10)} = 2.72$; between G1 and G3 $F_{(1,10)} = 1.99$; between G1 and G4 $F_{(1,10)} = 0.89$; between G1 and G5 $F_{(1,10)} = 3.55$; between G2 and G3 $F_{(1,10)} = 0.22$; between G2 and G4 $F_{(1,10)} = 0.49$; between G2 and G5 $F_{(1,10)} = 0.89$; between G3 and G4 $F_{(1,10)} = 0.05$; between G3 and G5 $F_{(1,10)} = 0.22$; between G4 and G5 $F_{(1,10)} = 0.06$ G1/G2/G3/G4/G5: 9.33 ± 1.03/10 ± 0.89/10.33 ± 1.21/10.5 ± 1.05/10.67 ± 1.03).The data in Table 3-14 are schematically presented in Figure 3-68.



The x axis was the group number, the y axis was the missing cell numbers.
There were no significant differences in the numbers of missing outer hair cell between the five groups, $p>0.05$.

Figure 3-68. The Numbers of The Missing Hair Cell in the Five Groups

Chapter 4 - Discussion

4.1. Research Question 1

The response patterns of DPOAE, ABR and CM are discussed. In DPOAE, the response pattern is the plots of the SNR of DPOAE at the measured frequencies. In ABR/CM, the response patterns are the plots of the amplitudes of ABR/CM at the measured frequencies.

4.1.1. The Response Pattern of DPOAE

An important contribution of this study to the research literature is the further development and verification of the technology to measure 0.5 kHz DPOAE successfully. To date, there have been a few reports about 0.5 kHz DPOAE. The measurements of 0.55 kHz DPOAE were reported in humans, and if the SNR $\geqq$ 6 was taken as the inclusion standard, the acceptable measurements of 0.55 kHz DPOAE were in the range of 42 to 66% (Beattie, 2003). In this study, acceptable 0.5 kHz DPOAE were recorded in all animals, with the minimum SNR of 0.5 kHz DPOAE being approximately 15.

For the normal response pattern of DPOAE in group 1, it was noted that, the SNR of DPOAE had a tendency to rise when the measured frequencies increased. There might be two explanations for this finding. The first explanation is that low frequency DPOAE is easily affected by the background noise, so the SNR of DPOAE is small at low frequencies. At high frequencies, the interruption caused by background noise was minimal, and the SNR of DPOAE increased correspondingly. This finding is consistent with previous research on the topic.

Oswald (2002) studied the relationship between DPOAE and behavioral thresholds and noted larger discrepancies when the relationships at 2 kHz and 8 kHz were compared to those at other

frequencies because the SNRs of DPOAE had a tendency to increase when the measured frequencies increased (Oswald, 2002). Siegel (2002) also reported that the SNR of DPOAE at 0.5 kHz was usually smaller than that at higher frequencies. Siegel found that because the SNRs of 0.5 kHz DPOAE were sparse, a reliable relationship between DPOAE and the behavioral thresholds could not be proved at this low frequency level.

As in Siegel's (2002) study, at high frequency more SNRs of DPOAE met the inclusion criterion and the correlation between DPOAE and the behavioral thresholds became stronger. The maximum value of Pearson Correlation Coefficients was 0.85 at 4000 Hz, with the value decreasing slightly at 6000 and 8000 Hz. At higher frequencies, the behavioral threshold could be predicted by DPOAE, indicating that the SNRs of DPOAE were robust at high frequency.

The second explanation for the normal response pattern of DPOAE is that the auditory acuity of guinea pig at high frequencies is better than that at low frequency. In guinea pigs the most appropriate frequency range of hearing was between 4 kHz and 20 kHz (Heffner, 2007). Thus, the SNR of DPOAE recorded at an appropriate hearing frequency (above 4 kHz) was higher than that recorded at the lower, less appropriate frequency range (0.5 kHz).

Because the frequency ratio between the two acoustic stimuli (*f2/21*) was very important for evoking an acceptable SNR of DPOAE, usually the ratio of *f2/21* was 1.2, it was not surprising that stimulus frequency might have effects on the SNR of the DPOAE (Abdala, 1996; Gaskill, 1990).

Since DPOAE is commonly used in the clinical setting, the successful recording of 0.5 kHz DPOAE will greatly expand its clinical application. When an individual had a history of noise

exposure, and the SNR of low frequency DPOAE is below the normal value, there might be a defect in the low frequency hearing. If a subtle change is found in the SNR of DPOAE after noise exposure, but there is no significant changes in the temporary threshold shift, and/or permanent threshold shift, this finding indicates sub-clinical cochlear damage, and DPOAE is more sensitive than temporary or permanent threshold shift (Attias, 1998, 2001; Desai, 1999; Plinkert, 1999). It was noted that if the SNR of DPOAE recorded before noise exposure was very high, there was a decreased risk of temporary threshold shift (Engdahl, 1996a). DPOAE might be used to monitor the dynamic changes of the cochlear damage induced by noise exposure, from temporary threshold shift to permanent threshold shift (Pankaj, 2015).

The contralateral suppression effect of DPOAE might be an indicator for the susceptibility to NIHL. An acoustic stimulation of one ear cochlea might also modify the cochlear activity of another ear (the contralateral cochlea). In this case, the SNR of DPOAE from the contralateral cochlea is typically reduced (contralateral suppression effect of DPOAE). Researchers reported a positive correlation between temporary threshold shift and contralateral suppression effect, such that the greater the suppression effect was, the more possibility the temporary threshold shift would occur (Engdahl, 1996b; Maison, 2000).

The mechanism underlying the generation of the DPOAE is not clear. Currently, the protocols of DPOAE are based upon empirical tests. Much more work is needed to understand the underlying mechanisms of DPOAE before it can be fully used in the clinic. Other important information is missing, including the dynamic observation of DPOAE during the development and treatment of NIHL. It is time consuming and expensive to obtain this information in animal experiments, and

it is even more difficult to do in humans. However, this information is important, as it will allow researchers and clinicians to know at which point of the development of NIHL the DPOAE has the best specificity and sensitivity, and how the DPOAE can be used to predict the prognosis.

4.1.2. The Response Pattern of ABR

In the current study, the smallest amplitudes of ABR were at 0.5 kHz. The first possible reason for this finding might be that the magnitude of ABR was dependent on the extent of neural synchrony; the less neural synchrony, the smaller amplitudes of ABR. When 0.5 kHz tone burst was used to evoke ABR, a partition close to the cochlear apex was stimulated, but the cochlea has little synchronous activity in this partition, so the amplitudes of 0.5 kHz ABR were small (Jerger, 1978). Thus, stimulus frequency had effects on the amplitudes of ABR (George, 1992). It was also found that the smallest amplitudes of ABR were at 0.5 kHz, with the increment of stimulus frequency, the amplitudes of ABR increased, achieving its maximum amplitudes at 8 kHz consistent with the most appropriate frequency range of hearing of the guinea pig (Heffner, 2007).

The response pattern of ABR found in the current study is consistent with previous research. In a study on normal hearing in adults, hearing thresholds estimated by pure tone audiometry (PTA) and the thresholds estimated by ABR were compared at four speech frequencies, 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. The thresholds estimated by PTA were relatively the same across the speech frequencies with less variability. However, thresholds estimated by ABR were not the same across the speech frequencies, probably as a result of the different magnitudes of ABR across the four speech frequencies (Schmulian, 2005). Thus, at high frequencies hearing loss could be underestimated, and at low frequency hearing loss could also be over-estimated. ABR performed

differently across the measured frequencies, and the measured frequencies were associated with the magnitudes of ABR (Purdy, 2002). When ABR is used to evaluate hearing function, frequency is an important reference factor before the data can be interpreted.

The successful measurements of low frequency ABR has expanded its clinical applications, particularly in the diagnosis of low frequency NIHL. Currently, NIHL is diagnosed and monitored primarily by PTA. PTA relies upon the subjective judgment and cooperation of the testee and the reliability of the subjective judgment can be questioned (McBride, 2003). Between 9–30% of noise-exposed workers exaggerate their hearing loss (Rickards, 1995), and there is a clinical need for an effective, objective hearing test to ensure correct diagnosis before treatment and perhaps compensation in medical/legal cases. An appropriate test needs to meet the following requirements, (a) it must be objective in both the recording procedure and the diagnosing standard (b) it must be appropriate to evaluate hearing function at both the low and high frequency.

Many electrophysiological measurements have been attempted to evaluate NIHL, for example, cortical auditory evoked potentials (CAEP) (Prasher, 1993), and the middle latency response (MLR) (Xu, 1996). CAEP is easily affected by the attention and the involuntary movement of the patient, such as blinking or chewing, there is greater variation in the amplitude of the waveform (Prasher, 1993; Squires, 1975). MLR could be evoked by frequency-specific tone bursts and used to evaluate NIHL (Xu, 1996). However, the narrow spectrum of the stimulus frequency and the decreased stimulus intensity may not evoke an acceptable synchronous neural firing, making it difficult to differentiate the MLR from the artefact (Goff, 1977). Also, the reproducibility of the

MLR needs to be improved (Wilson, 1987). Thus, ABR seems to be an appropriate option for the evaluation of NIHL.

Two kinds of stimuli could be used to evoke ABR, including click or tone bursts. Click evoked ABR primarily reflects hearing function in a narrow range of 2 kHz–4 kHz (Attias, 2006; Brookhouser, 1990), so the click evoked ABR might underestimate or miss hearing loss beyond that range, for example, very low and very high-frequency hearing losses (Picton, 1978; Pratt, 1978). One advantage of tone-burst evoked ABR is that it can be used to evaluate frequency specific hearing function, from low frequency to high frequency (Beattie, 1996; Johnson, 2005). Tone-burst was used in the current study because it could provide frequency-specific signals of ABR (e.g., 0.5 kHz ABR). In the current study, the signals of tone burst evoked ABR always had large positive amplitudes followed by low troughs, which indicated these signals were actual responses.

4.1.3. The Response Pattern of CM

In group 1, an important characteristic was noticed in the response pattern of CM: the highest amplitudes of CM were observed at 0.5 kHz. When stimulus frequencies went up, the amplitudes of CM went down, with the lowest amplitudes of CM at 8 kHz. Given that the stimulus intensities were the same, the amplitudes of CM evoked by higher frequency stimuli had smaller amplitudes than that evoked by lower frequency stimuli.

There are few reports about the mechanism regarding response patterns of CM. Zhang (2012b) has proposed several possible mechanisms about the response pattern. The first mechanism was that the response pattern of CM might be related to the electrical properties of the OHCs. In an

animal study (Palmer, 1986) it was found that there was a negative correlation between the amplitudes of CM and the stimulus frequencies. When the stimulus frequency was 0.5 kHz, the amplitude of CMs recorded from an intracellular electrode increased to approximately 12 mV. When the stimulus frequency was 5 kHz, the amplitude was about 1 mV, indicating that differences were likely a result of the electrical properties of the OHCs (Palmer, 1986).

The second possible mechanism is that the response pattern of CM is related to the property characteristics of the basilar membrane (BM). On the BM, at the partition around 0.5 kHz, when the length of this partition was divided by frequencies, the length per frequency was about 0.043% of the total length of the BM; at the partition around 6 kHz, the length per frequency was about 0.003% of the total length of the BM; so the BM's length per frequency at the partition around 0.5 kHz, is approximately 14 times longer than that at the partition around 6 kHz. A low frequency stimulus (0.5 kHz) could excite the longer partition of the BM greater than high frequency stimuli. Compared to the shorter partition of BM around 6 kHz, the longer partition of BM around 0.5 kHz might contain more OHCs which could generate larger amplitudes of CM. In summary, the numbers of OHCs in the partitions of cochlear apex were larger than that in the cochlear basal turns; the more OHCs, the larger the amplitudes of CM (Greenwood, 1990).

The third mechanism may be that the BM at the cochlear apex was wider and more flexible, whereas, at the basal turns the BM was narrower and stiffer. Thus, the displacement amplitudes of BM at the cochlear apex were expected to be greater than that at basal turns, contributing to the larger amplitudes of CM observed in the cochlear apex (Oghalai, 2004).

There is no doubt that the successful recording of CM across 0.5 to 8 kHz would greatly facilitate the clinical application of this technology. CM has been widely used to evaluate hearing function, including NIHL (Aran, 1976; Arslan, 1994; Eggermont, 1976). Recently auditory neuropathy spectrum disorder (ANSD) has been identified, and low frequency CM would be very useful in the differentiation between ANSD and cochlear diseases, particularly when low frequency hearing function is damaged.

ANSD is characterized by the impairment of central auditory system with preservation of peripheral auditory system (Berlin, 1998; Starr, 1996 and 2001). In ANSD, the impairment of central auditory system was the demyelination and axonal loss of auditory nerve fibers (Starr, 2001 and 2003), the damage of terminal auditory nerve dendrites, the loss of IHCs and/or their synapses with auditory nerve fibers (Starr, 1996; Starr, 2004). Correspondingly, the ABR was absent or abnormal in ANSD (Berlin, 1998; Rance, 1999; Starr, 1996, 2003 and 2004). In ANSD, the preservation of peripheral auditory system is indicated by the intact functions of OHCs, which is reflected by the presence of OAE and/or CM (Berlin, 1998; Deltenre, 1999; Rance, 1999; Starr, 2001).

It had been reported that OAE was not reliable in the diagnosis of ANSD, because it could not be detected in many ANSD patients, and OAE could by interrupted by middle ear disease. In some cases of ANSD, CM might be the only recordable measure of cochlear response (Deltenre, 1999; Rance, 1999; Starr, 2001). Thus, CM is an important diagnostic tool in the differentiation between ANSD and cochlear diseases (Deltenre, 1999; Rance, 1999; Sininger, 2001; Starr, 2001 and 2004). The absence of ABR and the presence of CM may indicate ANSD (Rance, 1999; Sininger, 2001).

The mechanism by which CM could reflect the functions of OHCs is not clear, and this limits its full clinical application (Withnell, 2001 and 2002). Although click-related CM is available clinically, measurement of frequency specific tone-burst evoked CM is not. If CM can be evoked by acoustic stimuli, it suggests at least a small functioning population of hair cells in the cochlea (Pedemonte, 2004; Tlumak, 2002). However, because the relationship between the amplitude of CM and the numbers of outer hair cells was not clarified, it is recommended that CM be used in combination with other auditory system tests, such as OAE (Dallos, 1971, 1974; Withnell, 2001).

In summary for Research Question 1, the response patterns of hearing tests provide an overview of hearing function across the measured frequencies. The response patterns are necessary for the evaluation of hearing function, because frequency is always a prerequisite reference in the interpretation of hearing tests. In normal hearing subjects, it is typical to have variability in the SNR of DPOAE, and in the amplitudes of ABR/CM across the measured frequencies. Compared with the response pattern of normal hearing, the patterns after noise exposures can indicate the frequency at which the hearing function is more sensitive to the damage of noise exposure, and specific effort can be made to prevent this hearing loss.

4.2. Research Question 2

4.2.1. 4 kHz Measurements

No differences were observed in the SNR of 4 kHz DPOAE, or in the amplitudes of 4 kHz ABR/CM. This finding showed the low/high frequency band-noises could only cause major damage to their corresponding partitions in the cochlea. Group 2 was exposed to low frequency

band-noises, and group 3 was exposed to high frequency band-noises; in group 3 the SNR of 4 kHz DPOAE or the amplitudes of 4 kHz ABR/CM were higher than that in group 2, indicating that low frequency band-noises could cause more damage to 4 kHz cochlear partitions than high frequency band-noises did. This finding is consistent with the knowledge that the movements of the basilar membrane induced by low frequency noise are usually greater than that induced by high frequency ones.

4.2.2. Effects of Single Band-noise Exposure

In group 1 (normal control), the SNR of 0.5 and 2 kHz DPOAE, or the amplitudes of 0.5 and 2 kHz ABR/CM, were significantly higher than that in group 2 (exposed to low frequency band-noises between 0.5 and 2 kHz). The SNR of 6 and 8 kHz DPOAE, or the amplitudes of 6 and 8 kHz ABR/CM in group 1, were significantly higher than that in group 3 (exposed to high frequency band-noises between 6 and 8 kHz). This finding suggests that a single band-noise exposure could cause damage to a specific partition in the cochlear basilar membrane. For example, the partition from 0.5 kHz to 2 kHz was sensitive to the band-noises between 0.5 kHz and 2 kHz, and the partition from 6 kHz to 8 kHz was sensitive to the band-noises between 6 kHz and 8 kHz.

4.2.3. The Effects of a Combination of Two Bands of Noise Exposure

In addition to the single band-NIHL, mixed hearing losses were generated by two band-noise exposures. In group 4, the first exposure was the low frequency band-noise (from 0.5 kHz to 2 kHz), and the second was high frequency band-noise (from 6 kHz to 8 kHz). In group 5, the first exposure was high frequency band-noise, the second was low frequency band-noise. At 0.5/2 kHz,

the SNR of DPOAE or the amplitudes of ABR/CM in group 4 or group 5 were smaller than those found in group 2. At 6/8 kHz, the SNR of DPOAE or the amplitudes of ABR/CM in groups 4 and 5 were smaller than those in group 3. Two band-noise exposure may cause more damage to hearing function than single band-noise, however the results were not statistically significant, $p>0.05$.

At 0.5/2 kHz, the SNR of DPOAE or the amplitudes of ABR/CM in group 4 were smaller than in group 5. At 6/8 kHz, the SNR of DPOAE or the amplitudes of ABR/CM in group 5 were smaller than in group 4. Again, the differences were not significant, $p>0.05$.

Researchers have previously reported similar results and argue that the first noise exposure could sensitize hair cells and increase susceptibility to the second noise exposure, causing severe hearing loss (Desai, 1999; Plinkert, 1999). The possible mechanism about the sensitization of hair cell is that noise exposure can increase the secretion of noradrenaline by the sympathetic nerve fibers that terminate close to the hair cells (Halperin, 2014), and the noradrenaline secreted from these adrenergic fibers, may sensitize cochlear hair cells (Aage, 2005).

The trends in our data are consistent with results reported in previous research, in that there seems to be an interaction between different types of noise. It may be that relatively high levels of noise intensity and longer durations of exposure are required to produce significant effects on hearing function (Fredriksson, 2015; Pekkarinen, 1995). In the current study, the maximum duration of noise exposure was about 2 hours, which is relatively short.

In group 4, after the first exposure of low frequency band-noise, some hair cells within the BM partition from 0.5 kHz to 2 kHz were damaged. Because the BM partition from 0.5 kHz to 2 kHz was sensitive to the first exposure of low frequency band-noise, the remaining hair cells might

be sensitized, and the susceptibility of the remaining hair cells to noise damage might be increased. In other words, the second high frequency band-noise could cause damage to the sensitized hair cells within the partition from 0.5 kHz to 2 kHz. Thus, there might be interaction between two noise exposures on the BM partition from 0.5 kHz to 2 kHz.

In group 4, the BM partition from 6 kHz to 8 kHz was not sensitive to the first exposure of low frequency band-noise, and the hair cells within this partition were not sensitized by the first exposure. The hair cells within this partition were damaged by the second exposure of high frequency band-noise, but there was no interaction between two noise exposures on the partition from 6 kHz to 8 kHz.

In group 5, the first exposure was high frequency band-noises, some hair cells within the BM partition from 6 kHz to 8 kHz were damaged by the first exposure, and the remaining hair cells were sensitized. When the animals were then exposed to the low frequency band-noises, there might have been an interaction between two noise exposures on the BM partition from 6 kHz to 8 kHz.

In group 5, the first exposure of high frequency band-noises might have little effect on the hair cells within the partition from 0.5 kHz to 2 kHz, and the hair cells within the partition would not be sensitized by the first noise exposure. The hair cells within this partition were damaged by the second noise exposure of low frequency band-noises, but there was no interaction between two noise exposures on the BM partition from 0.5 kHz to 2 kHz.

In group 4, there was an interaction between two noise exposures on the BM partition from 0.5 kHz to 2 kHz, but there was no interaction between two noise exposures on the BM partition

from 6 kHz to 8 kHz. In group 5, there was an interaction between two noise exposures on the BM partition from 6 kHz to 8 kHz, but there was no interaction between two noise exposures on the BM partition from 0.5 kHz to 2 kHz. Thus, the comparison between group 4 and group 5 showed that, at 0.5/2 kHz, the SNR of DPOAE or the amplitudes of ABR/CM in group 4 were smaller than that in group 5. At 6 kHz, the SNR of DPOAE or the amplitudes of ABR/CM in group 5 were smaller than that in group 4.

4.2.4. The Reduction in the SNR of the DPOAE and CM Amplitudes after Noise Exposure

In this study, group 1 was normal control, group 2 was exposed to low frequency band-noises, at 0.5 kHz and 2 kHz significant reduction in the SNR of DPOAE and the amplitudes of CM was observed in group 2. Group 3 was exposed to high frequency band-noises, and at 6 kHz and 8 kHz significant reduction in the SNR of DPOAE and the amplitudes of CM was observed in group 3.

The reduction in the SNR of 0.5 kHz or 2 kHz DPOAE of group 2, was smaller than the reduction in SNR of 6 kHz or 8 kHz DPOAE of group 3. This finding suggests that high frequency band-noises might cause more damage to the OHCs than the low frequency band-noises do. The findings of the current study are consistent with other studies in which DPOAE is affected by high frequency noise exposure (Büchler, 2012).

The reduction in the amplitudes of 0.5 kHz or 2 kHz CM of group 2, was larger than the reduction in amplitudes of 6 kHz or 8 kHz CM of group 3. This finding suggests that low frequency band-noises might cause more damages on the OHCs than the high frequency band-noises do, so smaller reduction in amplitudes of 6 kHz or 8 kHz CM were observed in group 3. The findings of

the current study are consistent with other studies, in which CM is found to be severely affected by low frequency noise exposure (Legouix, 1985).

In this study, reductions in the SNR of DPOAE and the amplitudes of CM were observed in the noise exposure groups. Both DPOAE and CM are generated by OHCs; IHCs can also contribute to the generation of CM. However, because the numbers of OHCs are much greater than the IHCs, it is believed that CM is mainly generated by OHCs (Withnell, 2001). And because noise exposure can induce the damage/death of OHC, it is expected that the SNR of DPOAE and the amplitudes of CM would be reduced after the noise-induced damage to hair cells. However, the mechanism about the damages of hair cell after noise exposure is still unclear (Patuzzi, 1989a).

The possible mechanism underlying the reduction in the SNR or the DPOAE and CM amplitudes after noise exposure is complex (Avan, 1993). Hair cell damage is often caused by noise exposure and ototoxins, such as aminoglycoside antibiotics (e.g. gentamycin) and antineoplastic agents (e.g. cisplatin). It was noted that caspase-9 (Yuan, 1999) and caspase-3 (Kirsch, 1999) were the key mediators in the damage/death of hair cell induced by exposure to noise, aminoglycosides, and cisplatin.

A group of pro-apoptotic and anti-apoptotic regulators in Bcl-2 family, such as mitogen-activated protein kinases (Zine, 2004) and p53 (Miller, 2000), could interact with the upstream promoters on caspase gene and control the level of gene transcription. Under certain physiological conditions, the inhibition of caspases gene could prevent the apoptosis of the hair cell. When hair cells are stimulated by noise exposure and/or ototoxic drug, the caspase gene is

activated, and apoptosis of the hair cell was accelerated; however, there are divergent theories in the regulating mechanisms (Wang, 2004).

After intense noise exposure, some hair cells may be damaged or die, so the numbers of functioning hair cell become smaller, and the SNR of DPOAE or the amplitudes of CM are reduced correspondingly. The remaining functioning hair cells might be less efficient in energy transduction, and the transformation between mechanical and electrical energy. A less efficient transduction could also lead to the reduction in the SNR of DPOAE or the amplitudes of CM. Some research indicates that noise exposure could cause abnormal changes in the potassium gating system, and in the end the transduction becomes less efficient (Holton, 1987; Patuzzi, 1989a).

4.2.5. The Reduction in the Amplitudes of ABR after Noise Exposure

The entire auditory system could be damaged after noise exposure, not only the peripheral cochlear could be affected, but also the central part of the auditory system could be injured. If a shift in the hearing threshold was seen after cochlear damage induced by noise exposure, it could be a temporary threshold shift, or a permanent threshold shift. After intense noise exposure, the damage to the auditory central nervous system was reflected as either reduced activity at many levels in response to auditory stimuli, from the lower level of cochlear nucleus to the upper level auditory cortex (Syka, 1989 and 2002), or increased neural sensitivity (hyper-sensitivity) and hyper-responsiveness to auditory stimuli.

After noise exposure, abnormal neural activity was seen in the cochlear nucleus and the inferior colliculus, their firing rates were reduced when the stimulus intensities were near, but

below the threshold; their firing rates were abnormally increased when the stimulus intensities were just above the threshold (Kaltenbach, 1998; Wang, 2002).

ABR has five peaks, P1–P5. It is generally accepted that each of five ABR peaks is composed of evoked potentials generated from multiple nuclei and tracts in the auditory central nervous system, primarily on the brainstem; and each of the auditory nuclei and tract could produce potentials that participated in several ABR peaks. P1 was mainly generated by ipsilateral cochlear nerve and cochlear nucleus. P2 was produced by the ipsilateral anteroventral cochlear nucleus (AVCN) and the posteroventral cochlear nucleus (PVCN). P3 was attributable to the AVCN, PVCN and the contralateral superior olivary complex (SOC). P4 was mainly generated by the AVCN. P5 was produced by the inferior colliculus (Kaga, 1997; Melcher, 1996). Noise damage could increase the amplitudes of middle latency responses (MLRs) recorded from the auditory cortex, but reduce the amplitudes of early latency responses, such as ABR (Møller, 1986; Syka, 2000).

Reductions in the amplitudes of ABR P5 were observed in the noise exposure groups, which meant the amplitudes of ABR P5 were sensitive to noise damage. These results are consistent with other studies (Nordmann, 2000; Ruttiger, 2013), however, the mechanism about the reduction in the amplitudes of ABR is poorly understood.

One possible mechanism is that the brainstem activity is regulated by two kinds of systems, an exciting system and an inhibiting system, and the amplitudes of ABR were determined by the balance between the two systems. The activity of the exciting systems might be down-regulated

by noise exposure, causing imbalance between the two systems and increased inhibition, thus reducing the amplitudes of ABR (Lim, 2005; Yang, 2007).

While DPOAE and CM are related to the function of OHCs, ABR seems to be related to the activity of IHCs. In previous research, the reductions in the amplitudes of ABR correlated well with the damage to IHCs and nerve fibers (Nordmann, 2000). The possible reason may be that the neural impulses of IHCs were reduced by the noise damage, and the adaptation of the reduced output (neural impulses) by the central auditory system was also compromised and became inefficient after noise exposure (Ruttiger, 2013).

4.3. Research Question 3

In the current study, CM EC significantly correlated with the CM RW in the presence of normal hearing and NIHL, and the essential characteristics of the CM waveform that are indispensable for diagnosis were preserved by ear canal recording, lending support to its clinical usage. Our result is similar to those reported by other researchers (Eggermont, 1976; Ferraro, 2007; Al-momani, 2009; Riazi, 2008). Up to now, there were no publications in which CM RW and CM EC were recorded in the same subjects, and then compared, as in the current study.

Recently there has been great interest in the non-invasive recording of CM, such as ear canal recording, mastoid recording and concha recording (Zhang, 2014 and 2015). In terms of the reflection of hair cell function, RW recording of CM is proposed to be the most sensitive of all available methods, and CM recorded at the round window is more likely to yield the best SNR

(Elberling, 1973). However, round window recording is too invasive to be routinely used in clinic (Fitzpatrick, 2014).

In the ear canal recording, the primary electrode is placed on the skin surface that is close to the tympanic membrane (Eggermont, 1976; Riazi, 2008). Although the amplitude of CM measured by ear canal recording was about 4-10 times smaller than that by trans-tympanic recording, ear canal recording could be easily performed by an audiologist, and it does not require a topical anesthetic as in trans-tympanic recording (Eggermont, 1976; Al-momani, 2009 Ferraro, 2007; Riazi, 2008).

In terms of clinical implications of these findings, two issues are noted. The first is related to the SNR of the CM. CM is typically detectable by trans-tympanic recording in the patients with SNHL, it was even detectable in the patients with severe SNHL, thus, CM can be detected by the trans-tympanic recording in the presence of extensive loss and/or damage of OHCs (Aran, 1976; Arslan, 1997; Schoonhoven, 1999). If CM cannot be detected in the patients with SNHL by non-invasive recording, it does not mean there was no CM generated by the OHCs. The lower SNR in the non-invasive recording might be reason, and modification should be made to the recording techniques to either increase the magnitude of CM or reduce environmental interruption (Sohmer, 1976 and 1980).

The second issue relates to the differentiation between CM and artefact. If the SNR is low in the EC recording, audiologists may need training in interpreting the responses of CM as CM can be confounded by artefact. For example, CM is a stimulus evoked potential, and this potential mimics the pattern of the stimulus waveform; however, the stimulus itself can be one of the

artefacts, so experience is needed to differentiate between CM and stimulus artefact. There may be many sources of artefact, but the two main sources are electrical interference from the transducer (speakers) and the vibration of the electrodes.

Any dysfunctions in the efferent tracts of the central nervous system might be confounding factors in the analysis of CM, because any changes in the function of the efferent nervous system could alter the electrical activity of OHCs, and affect the cochlear status (Guinan, 1996). It has been noted that the some diseases of central nervous system could cause the dysfunction of medial olivo-cochlear (MOC), and abnormal CM would present as a clinical sign (Hurley, 2002; Khalfa, 2001 a and b; Muchnik, 2004).

4.4. Research Question 4

Several researchers have attempted to correlate the reduced responses of hearing tests and the changes in the histopathology of hair cells after noise exposure (e.g., the relationship between the reduction in the SNR of DPOAE and the numbers of missing OHCs). However, the results from these studies have been contradictory. Results have ranged from no relationship between the reduction in the SNR of DPOAE and the numbers of missing OHCs, to a high correlation between them (Canlon, 1995; Hamernik, 2000; McFadden, 1998; Subramaniam, 1995). In the current research, there were no significant differences in the numbers of missing OHCs as a function of different types of hearing loss consistent with Subramaniam (1995).

Chen (2003) reported that no hair cell loss in the apical turn of the BM within 35% from the apex, which corresponds to frequencies less than 8 kHz. In the current study, the highest noise

frequency was 8 kHz, and there was no significant hair loss after noise exposure. In another research study (Cappaert, 2001), animals were exposed to broadband noises (40 Hz–40 kHz) at 95 or 105 dB SPL for 8 h/day, and total of 5 days, and it was found out that noise exposure could have significant effects on the physiological functions of cochlea, which were reflected in the changes of hearing test, but only a minor loss of OHCs in the first row. The functional loss without simultaneous hair cell loss is in line with other studies (Cappaert, 2000; Engstrom, 1984; Borg, 1995; Lataye, 1997 and 2000).

The simplest explanation for this apparent discrepancy between changes in test results and the loss of OHCs is that sub-cellular damage, e.g., molecular changes in the hair cells, such as the formation of mitochondrial free radicals, and molecular changes might not be detectable with the histological techniques. However, there might be some arguments about the pattern of hair cell loss. It was reported that in drug-induced SNHL, and in presbycusis, the third row of OHCs were affected and lost first; then, with hearing loss getting worse, the loss of OHCs expanded to the second and first row (Cappaert, 2001). There can be a relatively large loss of OHCs in the third row without any functional consequences. In NIHL, the first row of OHC is affected first. The first row of OHCs is the most important for cochlear sensitivity, the third row was relatively unimportant for cochlear sensitivity (Cappaert, 2001).

Another explanation for the lack of loss of OHCs following noise exposure in the current study is that the experiment was one of acute noise exposure, and the animals were euthanized immediately after the experiments. Single band-noise exposure was one hour, two band-noise exposures were two hours, so the maximum noise exposure was two hours. The hearing tests took

about one hour, so the animals were euthanized about one hour after noise exposure, and the whole experiment lasted about three hours.

Nordmann (2000) found that temporary threshold shift developed quickly after noise exposure, typically within three hours. Within this three hours, the damaged hair cells would undergo some histopathological changes, and the changes would evolve in two directions: some of the damaged hair cells would begin to recover within the three hours, and some of them would die. If most of the damaged hair cells recovered, the temporary threshold shift would resolve. The major histopathological changes during the period of temporary threshold shift were the buckling of the pillar bodies and hair cell swelling. If most of the damaged hair cells died, permanent threshold shift would develop, which would take place a minimum of 3 or 4 hours after noise exposure. The histopathological changes during the period of permanent threshold shift are the loss of hair cells. In the current study, the NIHL effects were at the stage of temporary threshold shift and the major histopathological changes during the period of TTS were the buckling of pillar bodies and hair cell swelling, not the loss of hair cells.

4.5. Technical Limitations

There remains disagreement about the source of the CM. For example, CM evoked by low frequency signals may not be restricted to low frequency regions of the basilar membrane, and that when low frequency stimuli are used in RW recordings of CM, both the OHCs at the cochlear apex and basal turn can contribute to the generation of CM, and the basal OHCs may even contribute more to the CM waveform because they are close to the recording electrode.

In his paper, Patuzzi (1989b) argued that low-frequency CM (0.1 kHz to 2 kHz) were measured in guinea pig cochlea before and after a variety of manipulations of the cochlea. These manipulations included the removal of the cochlear apex and the disabling of the cochlear basal turn. After the removal of cochlear apex, the low frequency CM could still be recorded. After the disabling of cochlear basal turn, the low frequency CM could not be recorded. Thus Patuzzi (1989b) concluded that that low-frequency CM was mainly generated by the OHC at cochlear basal turn.

There are two concerns about this paper. First, after the removal of cochlear apex, the low frequency CM could still be recorded; however, how it could be proved that this recordable-CM was not generated by the cochlear second turn? Second, after the disabling of the cochlear basal turn, the low frequency CM could not be recorded. Was the basilar membrane still functioning properly after the disabling of the cochlear basal turn? If not, how could the low frequency stimuli reach the cochlear apex? More evidence is needed to support Patuzzi's (1989b) conclusions.

If it is true that low frequency CM is mainly generated by the OHCs at cochlear basal turn, and basal OHCs are normal, the evaluation of low frequency hearing function would be difficult. In future studies, one possible solution is to use a low-frequency pure tone embedded in high-pass masking noise. Depending on the cut-off frequency, the high-pass masking noise should limit the contribution of basal OHCs to the CM and the contributions of apical OHCs would be clarified.

4.6. Conclusions

Low frequency SNHL is often seen in Meniere's disease (Enander, 1967), and people exposed to low frequency noises at their workplace (Zare, 2015). Low-frequency only SNHL is rare in the presence of intact high frequency hearing (Terkildsen, 1980; Thornton, 1980). Therefore, the low frequency specific hearing loss model in animal models is an appropriate option for the investigation of low frequency hearing function.

In humans with low frequency SNHL, objective auditory function tests are recommended for the evaluation of low frequency hearing. In current clinical practice, ABR, CM and DPOAE are commonly used, but there are some difficulties with the measurements of low frequency hearing tests, especially 0.5 kHz DPOAE and 0.5 kHz CM. In terms of the major contributions of the current research, a primary one is the successful recording of low frequency hearing tests through optimization of stimuli and equipment. In particular, 0.5 kHz DPOAE were recorded with the time averages of 32, and the 0.5 kHz DPOAE were recorded in all animal ears, with SNR above 15 dB SPL. Thus, our recording of 0.5 kHz DPOAE was easier and faster than that reported in previous research. Furthermore, the three tests were obtained in both normal hearing and in NIHL. Another important contribution of this study is the clarification of the relationship between CM RW and CM EC. Finally, from a technical standpoint, the successful animal experimental protocols in which the all details have been provided, may be a useful template for future research in animals and humans.

The current study has added more valuable information to the literature on the topics of low frequency hearing tests, and provided more reliable recording technology that can be used in the

clinic. As Parving (1982) pointed out, it is necessary to combine a variety of hearing tests to investigate the nature of low frequency hearing loss. This study included all three tests for a comprehensive perspective on the issue of measurement of low frequency hearing loss.

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