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Excessive exposure to more or less intense noise for longer duration could lead to elevation of hearing thresholds, which can be permanent or transient in nature. Noise induced temporary elevation in hearing thresholds is called temporary threshold shift (TTS). Recent animal studies have shown that noise induced temporary elevation in hearing thresholds are associated with loss of synaptic connections to the inner hair cells (IHCs). Two human studies have shown association between reduced ABR wave I amplitude and increased noise exposure history despite of normal hearing. The reduced ABR wave I amplitude is indicative of damaged synaptic ribbons and auditory nerve fibers with low spontaneous rate and high thresholds of IHCs. The purpose of the study was to identify difference in auditory nerve functioning between student musicians and non-musician students.

Methods: 75 collegiate students were recruited from a university campus and grouped into non-music major group (n=25), brass majors group (n=25), and voice majors group (n=25). All of the participants were screened for noise exposure using am online questionnaire. Participants were also screened for normal hearing and middle ear function using immittance and pure tone audiometry. ABR test was performed using two-channel setting for obtaining ipsilateral ABR responses with tiptrode and mastoid electrode simultaneously from left ear of each participant. The responses were evoked using click stimulus and presentation level begun at 90 dBnHL and decrease in 15 dB steps till 60 dBnHL. Amplitude of ABR wave I was calculated from the difference in

voltage at the positive peak and the voltage at the following negative dip for each participant.

Results: Tukey's test was utilized for group wise comparisons and the results showed significantly reduced suprathreshold ABR wave I amplitude in brass student musicians than non-musician students (p=0.0095). Voice majors group also showed reduced ABR wave I amplitude compare to non-musician (p=0.0428). The suprathreshold ABR wave I amplitude was not significantly different between voice students and student musicians playing brass instruments (p=0.8373).

Conclusion: The results of this study reveal that the normal hearing student musicians with brass instruments and voice exhibit reduced ABR wave I amplitude compare to non-musician students. This reduced ABR wave I amplitude is suggestive of damaged auditory nerve fibers with high threshold and low spontaneous rate. These fibers are crucial for detecting signal in presence of noise because they are resistant to masking. Ironically it is well documented fact that musicians outperformed non-musicians in tasks pertaining to perception of signals in presence noise. Intensive musical training, enhances subcortical and cortical structures underlying the neural encoding that are crucial for hearing in noise. These modifications in subcortical structures due to musical training might compensate for the peripheral damage of nerve fibers with high threshold and lower spontaneous rate. The biological mechanism which cause this subcortical modification is unclear and needs to be investigated.

INFLUENCE OF NOISE EXPOSURE BACKGROUND ON WAVE I AMPLITUDE ON STUDENT MUSICIANS AND NON-MUSICIAN STUDENTS

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Excessive noise exposure is believed to induce mechanical, metabolic, and neural changes in cochlea. Recent animal studies have shown that the tests of hearing threshold estimation are inefficient in identifying the subtle neurological damage in cochlea due to excessive noise exposure (Kujawa & Liberman, 2009; Lin, Furman, Kujawa, & Liberman, 2011; Furman, Kujawa, & Liberman, 2013). These neurological changes without any significant loss of peripheral hearing after excessive noise exposure might also occur in humans. In order to confirm this phenomenon in humans, there is need to study neural changes in the cochlea after noise exposure in individuals with normal hearing and history of noise exposure.

Noise induced hearing loss (NIHL) is one of the most common widespread disorder in industrialized countries. In the United States 23 million Americans between the ages of 20 and 69 years are estimated to have NIHL (Mahboudi et al., 2012). Approximately 22 million Americans are exposed to hazardous occupational noise annually (NIOSH, 2015). Factory workers, personnel who work on airport ramps or near operational aircrafts, and musicians are exposed to hazardous sound levels every day due to job requirements. Apart from occupational noise exposure, many individuals are exposed to higher sound levels during activities like listening to music, attending dance clubs, concerts, and playing video games at higher volumes (Clark, 1991).

Washnik, Phillips, and Teglas measured noise dose in collegiate student musicians and found that 49% of student musicians exceeded a 100% daily noise dose on at least one of two measurement days (Washnik, Phillips, & Teglas, 2016). Phillips, Henrich, and Mace (2010) reported early signs of NIHL in 45% of collegiate student musicians. Recent reports have shown that the risk of NIHL in children and adolescent has also increased. Shargorodsky, Curhan, Curhan, & Eavey (2010) analyzed the data of hearing thresholds in children reported in the National Health and Nutritional Examination Survey, 2005 and found that 19.5 % of the children aged between 12-19 years showed threshold shifts in one or both ears. Thus, the literature suggests that the impact of NIHL is not limited to populations who are exposed to higher sound levels due to occupation, but is a global hearing health issue (Niskar et al., 2001; Shargorodsky et al., 2010; Phillips et al., 2010; Barlow, 2011).

The adverse effects of excessive noise exposure can be categorized into non-auditory and auditory effects. The non-auditory effects of excessive noise exposure include sleep disturbances, stress, cardiovascular disease, endocrine disturbances and annoyance (Nelson, Nelson, Concha-Barrientos & Fingerhut, 2005; Stanfeld and Matheson, 2003). The auditory effects of noise exposure are tinnitus, hyperacusis, temporary threshold shift (TTS), and permanent threshold shift (PTS) which is also known as noise nduced permanent threshold shift (NIPTS). TTS and PTS are the most common adverse auditory effects of noise exposure (Heeringa & Dijk, 2014).

Temporary Threshold Shift (TTS) and Permanent Threshold Shift (PTS):

Excessive exposure to intense sounds for a sufficient duration can lead to elevation of thresholds of hearing after the termination of sound exposure. A PTS results when this elevation in threshold becomes permanent. PTS after noise exposure is a consequence of permanent damage to auditory structures. Damage to auditory structures causing NIPTS includes damage or loss of outer hair cells (OHCs) and inner hair cells (IHCs), damaged reticular lamina, swelling of stria vascularis, and loss of intermediate cells from stria vascularis, damaged or loss of auditory nerve fibers innervating hair cells (McGill & Schuknecht, 1976; Johnson and Hawkins, 1976; Bohne & Rabbitt, 1983; Bohne, Yohman, & Gruner, 1987; Wang, Hirose, & Liberman, 2002).

Temporary elevation in hearing thresholds due to an episode of sound exposure is called temporary threshold shift (TTS). Complete recovery from TTS may take a few minutes to a few weeks (Ward, 1970; Kujawa & Liberman, 2009). Recovery time for TTS varies from individual to individual and depends upon intensity, duration, and the frequency of exposure (Clark, 1991; Salvi & Boettcher, 2008). Nordman, Bohne, & Harding (2000) studied histopathological differences between TTS and PTS in Chinchillas. Animal's cochlea with TTS showed buckling of pillar bodies and loss of connection between stereocilia of OHCs and tectorial membrane in the region of TTS. In contrast, cochleas with PTS showed focal losses of both inner and outer hair cells and afferent nerve fibers at corresponding frequency region on basilar membrane.

One assumption underlying the concept of TTS is that after complete recovery from TTS, no residual anatomical or physiological damage is present, and the temporary

reduction in hearing is not associated with any deleterious physiological changes in the hearing system (Humes et al. 2005; Kujawa & Liberman 2009). Findings of the recent animal studies contradict this assumption (Kujawa & Liberman, 2009; Lin et al, 2011; Furman et al., 2013). In these studies, primary neuronal degeneration at the level of spiral ganglion and synapses between hair cells and auditory nerve fibers was observed in completely recovered ears from TTS after noise exposure. The results of these studies suggest that noise levels which were considered harmless may be deleterious, and exposure to such sound levels can cause permanent neuronal changes that can negatively affect auditory processing abilities (Kujawa & Libermann, 2009; Lin, Furman, Kujawa, and Liberman, 2011; Furman, Kujawa, and Liberman, 2013).

Results of all these animal studies indicate permanent damage to a certain proportion of auditory nerve fibers after recovery from TTS due to noise exposure. The findings of these studies need to be replicated in humans in order to apply this knowledge in developing therapeutic and preventive measures of NIHL. This line of research promises better understanding of impact of sound exposure on the human hearing system by unraveling the physiological differences between exposed and unexposed human ears to noise. In order to generalize the findings of animal studies to humans, new non-invasive studies in humans need to be conducted. Using Auditory Brainstem Response (ABR) as a tool, cochlear synaptopathy can be studied non-invasively in humans by targeting human populations who are frequently exposed to loud sounds such as musicians (professional and student musicians), industrial workers, railway workers and soldiers.

Collegiate student musicians are frequently exposed to hazardous sound levels. Barlow (2010) reported mean sound exposure level of 98 dB LAeq in undergraduate student musicians during rehearsals. Phillips and Mace (2008) also measured sound exposure levels in practice rooms of student musicians with different primary instruments. These researchers reported mean sound exposure level of 87-95 dBA for mean measurement period of 45 minutes. They also reported that mean exposure level of brass players was significantly higher than other instrument groups. Gopal et al. (2013) compared sound exposure levels during 50-minute jazz ensemble rehearsal and 50 minutes regular classroom activity (non-music), and found that the Leq (equivalent continuous noise level) during jazz ensemble ranged from 95 dBA-105.8 dBA, whereas in regular classroom activity the Leq ranged from 46.4 dBA to 67.4 dBA.

Gopal et al. (2013) also found significant temporary threshold shifts bilaterally at 4 kHz, and significant reduction in transient evoked otoacoustic emission (TEOAE) amplitudes bilaterally in student musicians after 50 minutes of a jazz ensemble rehearsal. Non-music major students did not show any significant difference in threshold and TEOAE amplitude after 50 minutes of regular classroom activity. Such a drastic difference in sound exposure level between student musicians and non-music students put student musicians at risk. A recent report by Stamper and Johnson (2015) showed evidence of noise induced cochlear synaptopathy in individuals with high noise exposure background. Considering the difference in exposure levels between music students and non-music major students it could be hypothesize that student musicians may exhibit signs of cochlear synaptopathy in spite of normal hearing. The objective of this study is

to investigate differences in cochlear and auditory nerve functioning between student musicians and non-musician students using wave I amplitude measurements from the auditory brainstem response.

CHAPTER II

REVIEW OF LITERATURE

Cochlear Physiology:

The primary organ affected by excessive noise exposure in the hearing system is the cochlea, and the damage caused by this excessive exposure is manifested in the form of TTS or NIPTS. The cochlea is the part of the inner ear which contains sensory end organ of hearing and is located in the petrous portion of the temporal bone. The snail-shaped cochlea has approximately 2.75 turns in human beings which contains 3 ducts separated by two membranes. Reissner's membrane separates the scala media from the scala vestibuli while the basilar membrane (BM) separates scala media from the scala tympani. The scala media is the middle duct, and it is filled with fluid called endolymph. The other two adjacent ducts, the scala vestibuli and scala tympani, are filled with another fluid called perilymph which is similar to extracellular fluid in ionic composition.

The scala media houses structures important to the transduction of sound, such as the basilar membrane, tectorial membrane, and stria vascularis. The organ of Corti on the basilar membrane has a specialized structure comprised of two types of receptor cells, the outer hair cells (OHC) and the inner hair cells (IHC), together with nerve endings and supporting cells. The inner hair cells are arranged in a single row and closest to the core of the cochlea. The OHCs are organized in 3-5 rows. Each OHC is mechanically coupled to a Dieters cell at the base and the reticular lamina at the apex.

The outer and inner haircells are responsible for the mechanical-chemical-electrical transduction (Kucuk & Abe, 1989) carried out by stereocilia bundles located at the top surface of these hair cells. The stereocilia bundles of IHCs are free standing at the apical end, and the stereocilia of OHCs are apically embedded in the gelatinous structure known as the tectorial membrane. A protein called stereocilin connects these stereocilia of OHCs to the tectorial membrane.

In one study, stereocilin knock-out mice exhibited the absence of waveform distortion and suppression of masking but unaffected cochlear amplification (Verpy et al., 2008). The findings of this study suggest that stereocilia bundles are responsible for the waveform distortion and frequency specificity of the BM. The mechanoelectric transduction channel through which K+ enters into the hair cell is located at the apical portion of the stereocilia. These stereocilia bundles are bathed by endolymph which has high K^+ concentration. The endolymph is at high positive potential compared to intracellular fluid of hair cells which has low concentration of K^+ and Cl^- (Zidanic and Brownell, 1990), and this difference in ionic concentration results in high potential difference between endolymphatic potential and intracellular fluid. The movement of endolymph due to sound energy deflects hair bundles away from the resting state, and this generates high electromotive force which drives K+ ions into the hair cells. The incoming K+ ions instantly exit from the hair cell passively through cell membrane (Johnstone, Patuzzi, Syka, & Sykova, 1989; Spicer & Schulte, 1998). This action is important for hair cells to achieve original functional state. The KCNQ4 channel located in the BM of hair cell is a major pathway for the departure of K^+ ions from hair cells.

The cycling of K+ ions is important for maintaining the endolymphatic potential. The K^+ released from the hair cells may be picked up by fibrocytes in spiral ligaments and transported from cell to cell through gap junctions into intermediate cells in the stria vascularis. These K^+ ions are released from the strial intermediate cells into the instrastrial spaces through the KCNJ10 channel that plays an important role in generating the endocochlear potential (EP). The basolateral membrane of strial marginal cells picks up the K^+ from intrastrial spaces through the $Na^+/2Cl^-/K^+$ cotransporter SLC12A2 and the Na^+/K^+ -ATPase ATP1A1/ATP1B2. Finally, strial marginal cell secretes K^+ back into the endolymph by KCNQ1/KCNE1 channel. (Wangemann, 2002).

As discussed earlier, the IHCs and OHCs rest on the BM which is tonotopically organized. The stiffness gradient throughout the BM makes it more responsive for higher frequencies at the base and lower frequencies at the apex. These hair cells convert the mechanical vibration of BM into an analogous electrical current which results in neuro-transmitter release (e.g., glutamate, acetylcholine) into the associated spiral ganglion neurons which consequentially activate the auditory nerve fibers. Spiral ganglion cells in the cochlea are of two types: 1) Type I auditory neurons (95%) are myelinated bipolar neurons with peripheral axons targeting the IHCs; 2) type II auditory neurons (5%) are small and unmyelinated neurons contacting only to OHCs (Spoendlin, 1972). The axon projections of both of these neurons connect with the central nervous system at the cochlear nucleus (Liberman, 1980). Each IHC is contacted by 10-30 auditory nerve fibers through a single synaptic ribbon to receive the neurotransmitter release (Furman, Kujawa, and Liberman, 2013). It has been suggested that the response phase of synaptic ribbons is

independent of stimulus intensity and this property facilitates the coding of frequency (phase locking), time, and intensity related to the acoustic features of stimulus. A reduction in spiral ganglion cells has been associated with NIHL, presbycusis, auditory neuropathy, and vestibular schwannoma (Kujawa and Liberman, 2009; Furman, Kujawa, and Liberman, 2013; Sergeyenko, Lall, Liberman, and Kujawa, 2013; Trautwein, Sininger, & Nelson, 2000; Glastonbury et al., 2002). A reduction in spiral ganglion and nerve fibers impairs the ability to detect a signal in noise.

The remarkable dynamic range of human audition is possible through the cochlear amplifier mechanism. The core of the cochlear amplifier mechanism is the OHCs, which increases the amplitude and frequency selectivity of sound vibrations using electromechanical feedback. Brownell, Bader, Bertrand, & de Ribaupierre (1985) observed a shortening and lengthening of OHCs during the depolarization and hyperpolarization phase, respectively. This unique feature of OHCs was referred to "electromotility" and this electromotility is driven by protein prestin (Liberman et al., 2002). Ashmore et al. (2010) assessed 2 main mechanisms underlying cochlear amplification: a stereocilia-based active amplification process and electromotility of OHCs. They suggest that the electromotility of OHCs is a strong mechanism which injects power into the movement of the basilar membrane.

Effect of Noise Exposure on Cochlea:

Excessive noise exposure causes an overdriving of the above-described processes, resulting in numerous physiological changes in the cochlea which are manifested in the form of TTS and PTS. The majority of the cochlear physiological changes in noise-

induced TTS are reversible. On the other hand, cochlear damage in NIPTS is permanent and irreversible. Many cochlear changes in NIHL are similar to changes in presbycusis, such as loss of hair cells, loss of nerve terminals, and damaged stria vascularis. Schuknecht (1964) classified presbycusis into four categories: Sensory, neural, metabolic (strial), and mechanical. In a similar fashion, physiological changes due to excessive high level noise exposure can be grouped into three categories: Metabolic, mechanical, and neural. The physiological changes due to excessive noise exposure cannot be grouped into a separate sensory type because the metabolic and mechanical changes overlap with sensory changes. The description of three types of changes occurring in NIHL are discussed in the next section.

Metabolic Changes:

The metabolic changes in the cochlea include changes in stria vascularis, blood flow, ionic changes, and metabolic changes in hair cells.

1. *Stria Vascularis:* High level sound exposure can cause acute swelling of the stria vascularis which is typically associated with loss of the intermediate cells of stria (Wang et al., 2002). In animal studies it was found that swelling of stria vascularis due to noise exposure gradually disappears, the loss of intermediate cells remains permanent in PTS (Hirose, and Liberman, 2003). Intermediate cells play a crucial role in K+ cycling and the loss of intermediate cells results in shrinkage of the stria vascularis and a short term decrease in endocochlear potential (Ide and Moirmitsu, 1990). The changes in endocochlear potential (EP) can be permanent with very high level sound

exposure, and these changes in EP are limited to the region in cochlea with extreme hair cell and stereocilia damage.

2. *Ionic changes in cochlea:* For normal hearing, maintenance of ionic balance across the apical membrane of hair cells is crucial. As discussed earlier endolymph has high concentration of K^+ and the critical level of K^+ within endolymph is maintained by potassium ion cycling. The pathway of K^+ cycling is complex; it includes cycling through OHCs followed by fibrocytes in the outer sulcus region of the lateral walls, and ultimately back to stria vascularis (Spicer and Schulte, 1996). In one study, the loss of type II and IV fibrocytes were observed in the region with high OHCs damage due to noise exposure (Wang et al., 2002). Being an important part of intracellular K^+ cycling pathway, the loss of type II and IV fibrocytes could potentially affect the K^+ cycling within cochlea.

   Spicer and Schulte (1998) suggest another route of K^+ cycling from IHCs. This pathway includes discharge of K+ ions from IHCs passing through phalangeal, inner sulcus cells and interdental cells and finally picked up by stellate fibrocytes. The K+ from fibrocytes are passed through gap junctions to intermediate cells of stria vascularis. Damage to any part of K+ cycling pathway involving IHCs or OHCs might affect EP.

3. *Changes in blood flow:* One of the consequences of noise exposure is reduced blood flow in cochlea (Lipscomb and Roettger, 1973; Thorne and Nutall, 1987; Miller et al., 2003). The magnitude of change in cochlear blood flow is

influenced by the intensity and duration of noise exposure. (Perlman & Kimura, 1962; Shaddock, Hamernik, & Axelsson, 1985; Prazma, Vance, Bolster, et al., 1987; Yamane, Nakai, Takayama, et al., 1995; Lamm & Arnold, 2000).

The two contributing factors for noise-induced reduction in blood flow are reduction in blood vessel diameter and red blood cell velocity (Quirk, Avinash, Nuttall, & Miller, 1992; Quirk and Seidman, 1995). The metabolic homeostasis of the cochlea is significantly affected by reduced blood flow as it creates ischemia. Ischemia reduces the cochlear oxygen supply and as an outcome, the phosphorylation process in mitochondria becomes more inefficient. This inefficiency also results in increase production of reactive oxygen species (ROS). High levels of ROS in the cochlea damages OHCs and may lead to apoptotic or necrotic cell death.

4. *Oxidative stress in hair cells:* The organ of Corti has many metabolically active tissues which produce free radicals as a matter of normal functioning. The presence of one unpaired electron in its structure makes a free radical highly reactive with other molecules. Reactive oxygen species (ROS) are oxygen based free radicals, which includes superoxide anion, O_2^-, the hydroxyl radical $\cdot OH$, peroxynitrite (ONOO-) and hydrogen peroxide H_2O_2 (Figure 2). The antioxidant defense system is present in almost all cells to neutralize the harmful effects of ROS (Kopke et al., 1999). These damaging ROS molecules are actually a byproduct of a normal metabolic process. The

electron transport chain is one of the important parts of normal metabolic process in sensory cells of cochlea. This electron transport chain occurs in the mitochondria of OHCs, and it is one of the major sources of superoxide. The electron transport chain is comprised of a series of reactions where electrons move from one carrier to another in order to release energy for the synthesis of ATP. During the movement of an electron from carrier to carrier, superoxide is formed as an intermediate molecule (Henderson et al., 2006).

The electron transport chain of mitochondria uses a large amount of oxygen during noise exposure, which consequently creates large amounts of superoxide generated as a byproduct. Furthermore, continuous noise exposure causes increased influx of Ca^{+} into OHCs through voltage sensitive L type channels. The increased influx of Ca^{+} results in intracellular and intra-mitochondrial accumulation of Ca^{+}. The calcium overload in mitochondria also leads to production of nitric oxide and ROS. Thus, noise exposure leads to increased production of free radicals/ROS within cells in multiple ways.

The excessive amount of highly reactive superoxides then react with other molecules to produce higher levels of other types of ROS in cochlea. (Halliwell & Gutteridge, 1999). The elevated free radicals and ROS breakdown lipid molecules located in membranes of outer hair cells, which affects channels of the cellular membrane. This process of lipid breakdown is called lipid peroxidation. The lipid peroxidation occurring in the plasma membrane alters membrane permeability, which consequently disrupts hair

cell homeostasis. Malfunctioning of ion channels results in imbalance of intracellular ionic concentration. For example, Lipid peroxidation in plasma membrane results in high influx of Ca+ in noise traumatized hair cells and supporting cells (Fridberger et al., 1998; Lahne and Gale, 2008). This high influx of ions causes swelling and ultimately results in necrotic cell death

These elevated free radicals and ROS also can damage DNA and disrupt protein synthesis, affect DNA repair and transcription process, oxidize proteins, destroy or destabilize membranes, disrupts ionic balance, alter cyto-skeletal components, and ultimately triggers cell death either by oncotic or necrotic pathway (Halliwell & Gutteridge, 1999; McFadden, Ohlemiller, Ding, Shero, & Salvi, 2001).

Increased concentrations of ROS due to prolonged noise exposure is not limited to OHCs. Yamane et al. (1995) reported elevation in superoxide levels in marginal cells of stria vascularis along with empty strial capillaries after high intensity rock music exposure in guinea pigs.

Mechanical Changes:

1. *Reticular lamina*: The top layer of the organ of Corti is comprised of the apical structures of both types of hair cells, supporting cells, and apical membranes of hair cells (Bohne, 1976). The stiff reticular lamina plays a crucial role in the maintenance of hair cell homeostasis as it acts as a barrier that separates endolymph and cortilymph. Noise-induced structural anomalies in the reticular lamina are typically observed at two anatomical sites, the

cuticular plates of hair cells and the cell-cell juncture between hair cells and supporting cells. These deformities in the cuticular plate are usually associated with hair cell degeneration. The degenerated hair cells create phalangeal scars and holes in the reticular lamina (Bohne and Rabitt, 1983; Ahmad, Bohne, and harding, 2003). The damage to cell-cell junction is typically caused by mechanical stress due to high level exposure of an impulse noise or blast. Damaged reticular lamina due to excessive noise exposure also leads to excessive influx of K^+ into OHCs. This excess K^+ leads to acute swelling of OHC and consequently results in apoptotic or necrotic OHC death (Henderson, Bielelfeld, Harris, & Hu, 2006).

2. *Plasma Membrane*: The plasma membrane of hair cells is an important cellular structure which functions as a cell boundary and plays an important role in cell-cell adhesion, maintenance of intracellular homeostasis, and extra- and intracellular communication. Mechanical injury to the plasma membrane is a consequence of excessive motion of the BM during noise exposure, which results in stretching injury to plasma membrane.
3. *Stereocilia of OHCs and IHCs:* The transduction channels crucial for hearing are located in the tips and shafts of stereocilia. During depolarization, K+ and Ca+ enter into the hair cells through these channels. Noise exposure affects the permeability of protein transduction channels in the cell membrane covering the stereocilia (Patuzzi, 2002). Morphological changes in stereocilia after noise exposure includes fused, bent, collapsed, and even missing

stereocilia. Some studies have reported that stereocilia of the innermost row of OHCs are most susceptible to damage followed by IHC, stereocilia of second and OHC rows (Robertson, Johnstone, and McGill, 1980; Fredelius, Johansson, Bagger-Sjoback, and Wersall, 1987). In contrast, results of other studies indicate that the stereocilia of IHCs are more vulnerable to noise trauma than OHC stereocilia (Engstrom and Borg, 1981; Kaltenbach, Schmidt, & Kaplan, 1992; Chen et al, 2003). OHCs lose contact with the tectorial membrane post exposure due to bucking of supporting cells (loss of contact between stereocilia of OHCs and tectorial membrane) which results in loss of hearing sensitivity (Nordmann, Bohne, and harding, 2000). The detachment and reattachment of OHCs stereocilia to tectorial membrane might be partially responsible for TTS and TTS recovery (Patuzzi, 2002; Saunders & Flock, 1986).

4. *Pillar cells:* Pillar cells act as supporting cells for hair cells and they are of two types, inner and outer. The pillar cells provide strong structural attachment between reticular lamina and basilar membrane. High level continuous and impulse noise damages pillar cells (Salvi, Hamernik, & Henderson, 1979). Loss of pillar cells affects local impedance of vibration of the organ of Corti. Loss of pillar cells may also trigger loss of OHCs.
5. *Hair cells:* The extent of damage to hair cells depends on the intensity and duration of noise exposure. The hair cell damage due to noise in TTS is different from PTS. Noise-induced TTS causes an increase in size and number

of lysosomes and enlarged nuclei, particularly in OHCs, which is manifested in the form of swollen OHCs (Sataloff and Sataloff, 2005). Mechanical and metabolic damage to plasma membranes also contributes in the swelling of hair cells. In the case of PTS, severe structural damage is evident in the form of hair cell death. Bohne, Harding, and Lee (2006) identified three death pathways of OHCs in noise- exposed cochleas of chinchillas: (1) Oncotic death pathway characterized by swollen, pale staining cell with swollen nucleus, (2) Apoptotic death manifested in the form of a shrunken, dark-staining cell with pyknotic nucleus (i.e. a degenerative condition of nucleus marked by clumping of chromosomes, hyperchromatism, and shrinking of nucleus), and (3) A pathway characterized by cells with absent basolateral plasma membranes and a nucleus lacking in nucleoplasm. Direct mechanical changes result from the physical forces of sound and occur during periods of high level noise exposure. Direct mechanical insult can be detected in cochlea immediately after intense noise exposure in animals. During the course of noise exposure, metabolic disturbances are initiated with a cascade of damage which continues for up to two weeks after termination of noise exposure. The mechanical, metabolic and neural changes due to noise exposure rarely occur separately.

The degeneration of hair cells typically occurs in clusters causing hair cell lesions involving a few or a large group of cells, depending on the duration and level of noise exposure. The site of lesion in the cochlea after noise

exposure is associated with the frequency composition of noise. Noise comprised of high frequencies selectively damages basal portion of organ of Corti, while low frequency noise preferentially affects apical portion of organ of Corti. Harding and Bohne (2009) exposed 1-3-year-old chinchillas to 4 kHz octave band noise at a variety of levels and duration. They reported that cochleas with high level exposure showed focal lesions distributed over the basal half of organ of Corti. In contrast, the cochlea with moderate levels of exposure manifested focal lesions in the region of organ of Corti corresponding to 4 kHz. Thus, high noise level exposure is associated with increased spread of frequencies affected and extended hair cell damage.

Pathological and physiological observations in noise-exposed animals showed two levels of lesion potentiation differentiated by critical level of noise exposure (Erlandsson, Hakanson, Ivarsson, Nilsson, & Wersall 1980; Vertes, Nilsson, Wersall, Axelsson, & Bjorkroth, 1982). When noise exposure is below a critical level, the extension of hair cell lesions increases slowly. As the noise exposure exceeds a critical level, hair cell damage increases substantially. Erlandsson et al. (1980) examined cochleas of guinea pigs who were exposed to intense pure tones of 3.85 kHz with levels ranging from 102 to 120 dB SPL for 6 hours. They reported that the level of hair cell damaged remains almost unchanged (5-8% hair cell loss) as the exposure level increased from 102 to 117 dB SPL. However, with further increase in noise exposure level from 117 to 120 dB SPL, an increase in the level of hair cell

loss was observed. This critical level is also associated with duration of noise exposure. For a noise exposure of shorter duration, the critical level will be higher than noise exposure of longer duration (Erlandsson et al., 1980). Moreover, the degree of hair cell loss is also associated with frequency composition of the noise exposure. The rate of hair cell loss is greater in high-frequency noise exposure than low-frequency noise exposure (Erlandsson et al., 1980).

The biological mechanism behind this sudden increase in cochlear damage is not clear. Spoendlin (1976) suggested that the mechanism of damage shifts from metabolic to mechanical, once the noise exposure exceeds the critical level. Many studies have reported a change in mode of hair cell death with an increase in noise exposure levels (Hu, Guo, Wang, Henderson, & Jiang, 2000; Yang et al., 2004). To examine the morphological changes in the cochlea with increased noise exposure levels, Hu et al. (2000) exposed guinea pigs to narrow band noise centered at 4 kHz with levels at 110 dB, 115 dB or 120 dB SPL for 4 hours. Through morphological analysis of the exposed cochlea they reported that noise exposure of 110 to 115 dB SPL causes mild hair cell damage and that the mode of hair cell death was necrotic. At 120 dB SPL noise exposure levels, the hair cell damage increases dramatically with mode of hair cell death shifted from necrotic to apoptotic.

Although both types of hair cells (i.e. OHCs and IHCs) may degenerate, the OHCs of the basal portion of cochlea are most susceptible to damage and

lost first with broadband noise exposure inducing NIPTS. Loss of OHCs due to noise exposure results in elevated hearing thresholds accompanied with loss of frequency tuning. The typical pattern of early NIHL includes notch centered at 4-6 kHz with a depth of 20-30 dB. The observed notch is a consequence of loss of OHCs centered at a corresponding region of the basilar membrane.

Neural Changes:

The afferent and efferent fibers of the auditory nerve innervate the hair cells (Spoendlin, 1985). Afferent innervation of hair cells is comprised of myelinated nerve fibers (95%) from type I neurons and unmyelinated nerve fibers (5%) from type II neurons. The myelinated nerve fibers innervate the IHCs while unmyelinated nerve fiber innervates OHCs. Excessive noise exposure causes IHCs to release high amounts of glutamate into the synapses of type I fibers of the auditory nerve. Glutamate is an excitatory neurotransmitter that functions at the synapses of IHCs and auditory nerve fibers. High concentration of glutamate at these synapses overstimulate the glutamate receptors on the post-synaptic cells. The consequence of this overstimulation is glutamate excitotoxicity, characterized by swelling and rupturing of post synaptic cell bodies and dendrites (Spoendlin, 1971; Puel, Ruel, Gervais d'Aldin, & Pujol, 1998; Kandel, Schwartz, & Jessel, 2000). Some studies have reported swelling of nerve terminals at hair cell synapses without hair cell loss within 24 hr post-exposure in TTS (Spoendlin, 1971; Liberman and Mulroy, 1982; Roberstson, 1983).

Earlier it was assumed that after complete recovery from TTS there is no residual anatomical damage. Some studies have shown that neural changes like the swelling of synaptic cell bodies and dendrites are reversible (Pujol et al, 1993, Puel et al., 1998; Pujol and Puel, 1999). However, recent animal studies revealed permanent neural damage despite complete recovery of thresholds and this neural damage continued for months post-exposure (Kujawa and Liberman, 2009; Lin et al, 2011; Furman et al, 2013). These studies used Auditory Brainstem Response (ABR) wave I amplitude as a tool to reveal post-exposure subtle changes occurring at the synaptic level.

ABR and Its Usefulness in Identifying Noise-Induced Neural Damage:

Auditory brainstem response is an index of primary auditory function that occurs in the brainstem. These evoked potentials are recorded non-invasively from the scalp and indicate synchronous neural activity within VIII nerve, brainstem, and midbrain. It is evoked either by a series of clicks or frequency-specific tone pips/bursts presented through headphones. The typical waveform of ABR is comprised of five peaks (Wave I, II, III, IV, and V) and these peaks have different generators which are located between the cochlea and the brainstem. The first major wave in ABR is Wave I which arises from the activity in the auditory nerve close to cochlea. The next major component is wave III whose generators are located in cochlear nucleus and superior olivary complex. The last major and most prominent component of ABR is the wave IV/V complex generated in the lateral leminiscus/inferior colliculus. The amplitude of ABR peaks reflect the number of neurons firing while the latency of ABR peaks represent the speed of transmission.

ABR has been used widely for threshold estimation and differential diagnosis. For estimating thresholds, wave V is used because it is the most robust wave and sensitive to decreasing intensity. Thus, it correlates well with behavioral thresholds of a subject. For threshold estimation clicks or tone bursts are presented first at high intensities and subsequently at lower intensities. The intensity level below which wave V could not be identified corresponds closely with the behavioral threshold, particularly with high frequency pure tone thresholds. The application of ABR in differential diagnosis has changed over time.

ABR provides important information about functional integrity of the auditory nerve and brainstem pathway. It is a useful test in differential diagnosis of acoustic tumor, brainstem lesion or stroke, demyelinating diseases (multiple sclerosis), and head trauma, ABRs for differential diagnosis are typically performed at high intensity levels with a faster repetition rate. The diagnostic interpretation of ABR is based on latencies and amplitudes of component waves I, III, and V. It has been reported that the increase in ABR threshold following permanent hearing loss due to noise exposure correlates with the damage to IHCs and loss of nerve fibers (Nordmann et al., 2000; Harding, Bohne, & Ahmad, 2002).

Findings of other studies suggest that ABR threshold estimation is not a good representation of subtle and permanent loss of type I nerve fibers occurring after complete recovery from TTS (Kujawa and Liberman, 2009; Lin et al, 2011; Furman et al, 2013; Fernandez et al, 2015). Threshold responses of ABR are insensitive to diffuse loss of type I neurons as OHCs are functioning normally. The auditory evoked cochlear

responses such as ABR are not sensitive to diffuse degeneration because the number of responding type I neurons increases rapidly as sound level increases. However, a decrement in amplitude of wave I at suprathreshold level is an appropriate reflection of neural degeneration in ears recovered from TTS (Kujawa and Liberman, 2009; Lin et al., 2011). As discussed earlier, the high threshold subtypes nerve fibers (i.e. low SR and medium SR) contact the IHCs from the modiolar side while the low threshold high SR connects to IHCs from pillar side of the cells. The high threshold fiber subtypes are more vulnerable to noise than low threshold because of inefficiency in buffering Ca^{+} overload and scavenging glutamate. The high threshold subtype nerve fibers contribute 40% of the total type I fibers and selective loss of these fibers will not affect ABR thresholds but the suprathreshold amplitudes of ABR would be reduced. Thus, a decrement of ABR wave I amplitude at suprathreshold levels is a sensitive measure of primary neural damage caused by noise exposure.

Post-Exposure Neural Changes:

Kujawa and Liberman (2009) studied neural damage in the cochleas of CBA/CAJ male mice with temporary noise-induced threshold shifts. The mice (age=16 weeks) from the experimental group were exposed to octave band noise (8-16 kHz) at 100 dB SPL for two hours (256 times the safe levels recommended by NIOSH) and held without any treatment for different post-exposure times. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded from all mice, and compound action potentials from a subset of mice just before tissue recovery for histological processing. For quantification of neural damage, mice were intravascularly

perfused and the cochleas were removed for histological analysis. At 24-hours post exposure, the ABR and CAP measurements showed a 40 dB increase in neural response thresholds accompanied with smaller increase in DPOAE thresholds, indicating OHC dysfunction and additional contribution from neural damage. The ABR findings also revealed a typically upward spread of damage in terms of frequency. Histological analysis of mice cochleas 24-hour post exposure showed swelling in peripheral nerve terminals of auditory nerve fibers. Two weeks post-exposure elevated thresholds in ABR returned to normal, however reduction in the supra-threshold response of the ABR suggested neuronal loss in high frequency cochlear regions. At 12 kHz, where ABR measurements showed a smaller threshold shift, 80% amplitude recovery was observed whereas, at frequency 32 kHz where the threshold shift was largest, amplitude recovery was only 40% two weeks after exposure. In contrast, DPOAE findings at 2 weeks post exposure revealed complete recovery at all frequencies. These findings indicated neuronal loss at high frequencies with intact OHCs after recovery. Degeneration of pre-synaptic and post-synaptic components of IHCs throughout the basal half of cochlea was observed at all post-exposure times. The presynaptic ribbons in noise-exposed cochleas were reduced in number and the remaining ribbons were either large or displaced from their original position.

After 8 weeks post exposure, at 32 kHz the neural amplitude and ribbon counts were decreased by 60% and 50 % respectively. On the other hand, at frequencies with small threshold shifts, e.g.12 kHz, the neural amplitude and ribbon counts were decreased by 30% and 10 % respectively. The degeneration of ganglion cells post-exposure was

quite different from presynaptic ribbons. At 2 weeks post exposure, ganglion cell counts in 32 kHz region were within the normal range; however, after 1 year there was an increased delayed loss of ganglion cells in noise-exposed cochleas. After 2 years post-exposure, ganglion cell numbers were reduced to approximately 50% in the 32 kHz region indicating prolonged and slow degeneration of ganglion cells after the termination of exposure. These findings suggest that acoustic exposure causing reversible threshold shift can cause irreversible damage to synapses, nerve terminals, and ganglion cells in mice.

The above findings raised the question of whether the observed irreversible neuropathy induced by moderate acoustic exposure in mice occurs in other animals. Lin et al. (2011) attempted to answer this question by studying neural damage post-exposure in guinea pigs. All the female guinea pigs in this study were divided into two groups, a control group and an acoustic injury group. Each guinea pig was pre-screened for cochlear functioning using ABR and DPOAE measurements. The guinea pigs from the acoustic injury group were exposed to octave band noise (4-8 kHz) at 106 or 109 dB SPL for 2 hours. The hearing function of animals from this group was tested using ABR and DPOAE at different post-exposure survival times ranging from 24 hours to 6 weeks. A small sub-group of animals from the acoustic injury group was allowed to survive for two years in order to track slower neural degeneration as observed in the study by Kujawa and Liberman (2009). Tone pip ABR was used to measure hearing function at different frequencies. Histological analysis of cochlea was conducted at different post exposure times. The findings of this study replicated the findings by Kujawa and Liberman (2009)

in all aspects. This study also reported high temporary threshold shift at frequencies above the noise band after exposure to 106 dB SPL noise. TTS measured by ABR was greater than TTS measured by DPOAE. For the group of animals who were exposed to 106 dB SPL noise, thresholds had recovered to pre-exposure values at the 10^{th} day post-exposure on both DPOAE and ABR test. On the other hand, the group of animals who were exposed to 109 dB SPL (a doubling of intensity) showed incomplete recovery after 10 days. As observed in the Kujawa and Liberman study (2009), ABR wave I amplitude was reduced significantly in the noise-exposed group compared with controls despite complete recovery from TTS.

The results of the confocal imaging analysis correlated well with the previous study as this study also revealed substantial loss of the presynaptic ribbons of IHCs extended across the basal half of the cochlea. The ribbon counts were reduced up to 55% at 10 days post-exposure. The 2011 analysis also revealed swelling of afferent nerve terminals, which was only limited to IHCs (i.e., no swelling at OHCs). Larger or displaced synaptic ribbons beneath the IHCs corresponding to high frequencies were found in noise-exposed cochleas. The results of the analysis also showed slower ganglion cell loss 2 years post-exposure but the magnitude of ganglion cell loss was smaller when compared with the previous study. The morphological analysis of the cochlea revealed separation of ribbons. The high threshold subtype ribbons, low spontaneous rate (SR) and medium SR were on the modiolar side of IHC, while the low threshold high SR ribbons faced towards the pillar cells. The morphological analysis did not reveal any differential pattern of ribbon loss on the two sides of IHCs.

The findings of Kujawa and Liberman (2009) and Lin et al. (2011) have shown that TTS-inducing noise exposure is not benign in nature and may lead to irreversible neural damage. However, the results of the study do not provide any information about the cumulative effect of noise exposure inducing TTS. Wang and Ren (2012) studied the effect of repeated TTS noise exposure in CBA/Caj mice. All the animals entered into the study protocol were 4 weeks of age and exposed to octave band noise (12 kHz center frequency) at 100 dB SPL for 2 hours. These mice were divided into three groups randomly based on the number of exposure episodes. Mice from the first group were exposed only once at 4 weeks of age, mice from the second group were exposed to noise at 4 and 6 weeks, and mice from the third group were exposed at 4, 6, and 8 weeks of age. Animals from all three groups were given 2 weeks of time to recover from prior exposure. Hearing sensitivity was measured using ABR and DPOAE at 24 hours and 2 weeks post exposure. On comparing TTS 24 hours post exposure between these three groups using an ANOVA , the patterns of TTS in ABR were similar: with highest shift (i.e. 30-40 dB) at approximately 23 kHz ($p>0.05$) Complete recovery was observed 2 weeks post exposure in the first and second group but not in the third group. On pairwise frequency comparisons between three groups using ANOVA, the animals exposed to noise three times showed worsening of threshold at frequencies above 11 kHz 2 weeks after the third exposure ($p<0.01$).

The DPOAE findings were also similar, showing complete recovery after 2 weeks in animals exposed to noise once and twice ($p>0.05$), but not in animals who were exposed to noise three times. For the group of animals who were exposed to noise three

times, at 16 kHz no loss of DPOAE amplitude was detected ($p>0.05$), however some small reduction in DPOAE was observed at 22 kHz ($p<0.01$) and 32 kHz ($p<0.01$, with significant pairwise posthoc comparisons). The TTS measured at 23-32 kHz using ABR was greater than the amplitude reduction in DPOAE after the third noise exposure, suggesting that neural elements contribute more in TTS.

The amplitude of wave I in groups one and two were reduced by approximately 50% compared with the controls at all suprathreshold levels. On comparing the amplitude of wave I between group 1 and 2 using an ANOVA, no significant difference in the amplitude reduction was observed ($p>0.05$). In contrast, the third group showed additional wave I reduction of approximately 25% with PTS ($p<0.01$) compared to group one and two. In the morphological analysis, loss of synaptic ribbons was similar in groups one and two ($p>0.05$) while group three showed elevated synaptic ribbon loss ($p<0.01$, one-way ANOVA with post hoc pairwise comparisons). Only half of the ears with three episodes of noise exposure showed OHC loss at the cochlear base. The rest of the ears did not show loss of IHCs or OHCs. These morphological results do not show preferential loss of ribbons on the pillar and modiolar side of IHCs. Overall, this study showed a cumulative detrimental effect of noise exposure episodes, which was manifested in both physiological and morphological analyses.

The reduction of wave I amplitude at suprathreshold stimulus levels in animals without loss of hair cells suggests the possibility of selective auditory nerve fiber damage, which includes fibers with high thresholds and low SR. However, the studies discussed above (Kujawa & Liberman, 2009; Lin et al, 2011; Wang & Ren, 2012) did not find any

preferential loss of nerve fibers in TTS-induced ears. Furman et al. (2013) hypothesized the selective degeneration of auditory nerve fibers with high threshold and low SR in recovered ears from TTS. Single auditory nerve fiber responses were recorded from female albino guinea pigs who were exposed to OBN (4-8 kHz) at 106 dB SPL for two hours. The physiological tests, ABR and DPOAE, were conducted prior to noise exposure and two weeks' post-exposure. In addition, single auditory nerve fiber recordings were collected from control animals. ABR thresholds recovered completely but the suprathreshold amplitude of wave I was reduced two weeks post exposure. The DPOAE measurements (i.e. amplitude and thresholds) also showed complete recovery after two weeks. Quantitative synaptic analysis using 2-way ANOVA from a large sample of controlled and exposed cochlea revealed significant reduction in synaptic counts for cochlear regions with CF> 4kHz ($p<0.01$). On comparing single nerve fiber recordings from exposed and unexposed ears no significant difference was observed in frequency tuning, post onset adaptation, dynamic range, and first spike latency ($p>0.05$ by Kolmogorov-Smirnov). However, statistical analysis of single nerve fiber data revealed higher loss of low SR fibers in the region of noise exposed cochlea corresponding to high characteristic frequencies ($p<0.01$ by Kolmogorov-Smirnov).

Furman et al. (2013) reported that, in control ears, the low and medium SR fibers contributed to 47% of the total population of AN fibers innervating the IHCs with a CF>4 kHz, while in exposed ears the medium and low SR fibers comprised only 29% of the population. This disproportionate loss of low and medium SR fibers was not evident in the apical half of the cochlea.

Each IHC is contacted by a number of auditory nerve fibers, which are spatially segregated and differ in spontaneous rate (Liberman, 1978; Liberman, 1982). The high spontaneous rate fibers have the lowest threshold and tend to connect to the IHC from the side that is closer to pillar cell. The lowest spontaneous rate fibers have the highest threshold and medium spontaneous rate fibers have an intermediate threshold. Both of these types of fibers make contact to IHC from the modiolar side. This difference in spontaneous rate of AN fibers innervating IHCs plays an important role in extending the dynamic range of peripheral hearing system. The low SR fibers have high thresholds, which makes them more resistant to masking by continuous background noise (Costalupes et al., 1984). Typically, we hear with high SR fibers in quiet situations but as the background noise increase humans rely heavily on low SR fibers. Thus, loss of low SR fibers might affect sound perception in noisy situations.

The low SR neurons are more vulnerable to damage during acoustic exposure. These neurons first disappeared in acoustically traumatized cats (Liberman and Kiang, 1978) and guinea pigs (Furman et al., 2013). There are two possible mechanisms which make low SR fibers more vulnerable to damage after noise exposure. The first mechanism is related to glutamate excitotoxicty. Continuous noise exposure results in excessive glutamate release from IHCs. The excessive glutamate released from the IHCs needs to be scavenged effectively otherwise it will lead to excitotoxic manifestations in the form of swelling of post synaptic cell bodies and dendrites (Kandel, Schwartz, and Jessel, 2000). Furness and Lawton (2003) suggested that the scavenging process of glutamate is less efficient on the modiolar side than pillar side of IHCs and it is the

modiolar side where the low SR fibers are predominant. The other possible mechanism is associated with fewer mitochondria in low SR fiber than high SR fiber terminals (Liberman, 1980). Due to fewer mitochondria, the low SR fibers are not capable of buffering the Ca+ overload which is important in generation of glutamate excitotoxicity (Szydlowska and Tymianski, 2010).

Studies have reported delayed and slowed degeneration of ganglion cells which continues for 1-2 years (Kujawa and Liberman, 2009; Lin et al., 2011). Considering the frequency of such episodes of noise exposure and the delayed neural degeneration, it has been hypothesized that the neural changes occurring after complete recovery from TTS influences age related hearing loss. Fernandez, Jeffers, Lall, Liberman, and Kujawa (2015) examined cochlear aging after two types of noise exposure. One exposure induced permanent synaptic damage without hair cell loss, while another exposure neither induced synaptopathy (i.e. loss of synapses) nor hair cell loss. The CBA/Caj mice were divided into three groups. The mice from group 1 (i.e. synaptopathic exposure group) were exposed to 8-16 kHz OBN at 100 dB SPL for 2 hours. The mice from group 2 (i.e. non synaptopathic exposure group) received exposure of the same frequency at 91 dB SPL. The group 2 were subdivided into 2a and 2b. The mice from group 2a received exposure for 2 hours while group 2b received exposure for 8 hours. The reduced level of noise exposure in group 2 did not cause any acute synaptic loss. Group three was the control group which was comprised of age-matched unexposed animals. Mice from all the groups were evaluated at different post exposure times which ranged from 1 hour to 20 months. Cochlear function of the animals was evaluated using DPOAE and ABR. To

quantify HCs, cochlear neurons, and synapses, cochlear whole mounts and plastic sections were examined. The synaptopathic group showed a 35-50 dB threshold shift 24 hours post exposure and recovered completely by 2 weeks. However, synaptic counts and wave I ABR amplitude at suprathreshold levels were reduced by approximately 45 %. Moreover, animals from the synaptopathic group showed elevated synaptic loss and exacerbated OHC loss with aging compared to controls. On the other hand, animals from the non-synaptopathic exposure group exhibited transient threshold shift without acute synaptopathy. They also showed no synaptic loss or cochlear dysfunction up to one-year post exposure. Two important things can be concluded from this study. First, not all noise exposure episodes inducing TTS causes synaptopathy. Second, single synaptopathic exposure can boost age related degeneration in the cochlea.

All the animal studies discussed so far clearly indicated that cochlear synaptic loss persists even after complete recovery from TTS. A similar experiment cannot be conducted in humans for ethical reasons. However, it is important to answer the question as to whether humans also show signs of noise induced damage at the neural level. Stamper and Johnson (2015) attempted to answer this question in their study by recruiting 30 normal hearing human subjects (age= 19-28 years) with different noise exposure backgrounds (NEB). The NEB of each participant was quantified using Megerson noise exposure questionnaire (Megerson, 2010) which inquired about loud sound exposure in the last year. Cochlear functioning was evaluated using DPOAE and ABR. DPOAEs were measured for f2 frequencies of 1, 2, and 4 kHz. The presentation level of DPOAE began at 80 dB FPL and decreased in 10 dB steps until 0 dBFPL. Two

channel ABR was collected using clicks and 4 kHz tone bursts as stimuli presented at a rate of 11.3/sec with alternating polarity. One of the two channels used an ipsilateral tympanic membrane electrode, while the other channel used an ipsilateral mastoid electrode. The presentation level started at 90 dBnHL and decreased in 10 dB steps until 10 dB below threshold.

The data in this study were collected in two sessions. All the participants were asked to avoid participation in any noisy activity the night before the first and second sessions. The first session comprised of consenting procedures, audiometric evaluation, and assessment of noise exposure background. DPOAE and ABR were recorded in the second session. The results of the study revealed a statistically significant correlation between NEB and ABR wave I amplitude at suprathreshold levels recorded using a mastoid electrode ($p=0.015$, $r^2=0.194$). A systematic trend of decrease in wave I amplitude with increase NEB was found at level 90-70 dBnHL. This trend weakened and gradually disappeared below 60 dBnHL stimulus level. Although ABR using TM electrode showed similar patterns, no significant relationship was found between wave I amplitude and NEB ($p=0.095$, $r^2=0.097$). The r^2 value indicates that approximately 20% of variance in wave I amplitude was explained by NEB. Furthermore, no systematic relationship was found between NEB and suprathreshold DPOAE and ABR wave V amplitude. The results of this first human study were consistent with findings from animal studies and it suggest that a similar mechanism of noise-induced reduction in wave I amplitude as observed in animal studies might be observed in human ears

Research Hypotheses and Rationale:

The study by Stamper and Johnson (2015) showed an association between increased NEB and reduced amplitude of ABR wave I. However, this association was observed within a single group. These findings could not be generalized because the association was observed within a single group where the NEB varies from participant to participant. Furthermore, the NEB was obtained by taking the median values of the exposure level ranges of different types from previous literature. In the current study we examined the wave I amplitude of ABR in student musicians (i.e. brass players and vocalist) and non-music students. The music students were our experimental population because previous research work showed that 49% of student musicians exceeded 100% noise dose on one of two measurement days (Washnik et al., 2016). The student musician groups and the non-musician group were controlled in terms of noise exposure other than exposure through musical instruments.

The findings of the current study were expected to show reduced amplitude of ABR wave I in student musicians when compared with non-music students. The results of the current study confirm the existence of loss of selective auditory nerve fibers loss in exposed humans. This study also provided preliminary knowledge of a neural basis of NIHL which has not been studied in detail so far in humans. The results of this study will be useful in genetic association studies in NIHL particularly related to glutamate excitotoxicity as the previous literature suggest that the high threshold, low spontaneous rate nerve fibers are more vulnerable to damage due to inefficient glutamate scavenging.

Hypothesis 1: Student musicians with brass instruments will exhibit a reduced amplitude of wave I ABR at suprathreshold levels when compared with non-music major students.

Rationale for Hypothesis I: Student musicians with brass instruments are exposed to hazardous sound levels during individual practice and group rehearsals (Phillips and Mace, 2008; Barlow, 2010). This high level music exposure induces TTS (Strasser, Irle, and Legler, 2003; Toppila, Koskinen, & Pyykkö, 2011; Gopal et al., 2013). Thus, student musicians with brass instruments are exposed to higher sound exposure \levels several hours every day than typical non-music major students due to exposure music exposure through musical instruments. Higher sound exposure levels inducing TTS has been shown to cause permanent damage to nerve fibers despite complete threshold recovery in animals and this damage is manifested in the form of a reduced amplitude of ABR wave I. Thus, it is expected that loud sound exposures producing TTS may cause similar neural damage in humans, as observed in animals. A recent study by Stamper and Johnson (2015) also found reduced amplitudes of wave I ABR in individuals with a high noise exposure background compared with individuals with a low noise exposure background. This reduced amplitude suggested loss of nerve fibers innervating IHCs in cochlea. Many of the subjects in this study with high noise exposure backgrounds were music students. In one of the survey study, only 29% of the students reported working in noisy environment and 50 % of the students reported listening to music through headphones at moderately high to high levels (Rawool & Collogon-Wayne, 2008). This study by Rawool and Collogon-Wayne (2008) indicates that a large portion of collegiate students have lower sound exposure. Thus, we expected that student musicians with

brass instruments will show reduced wave I amplitude due to their high noise exposure background when compared with non-music major students selected for a low noise exposure background.

Hypothesis 2: The amplitude of ABR wave I will be lower in student musicians of voice than non-music students.

Rationale: Among student musicians the noise exposure varies based on the primary instruments. Phillips and Mace (2008) showed that average sound levels in student musicians with brass, woodwind and percussion instrument are significantly higher than student musicians of voice while practicing in music rooms. However, these voice student musicians also attend ensemble rehearsals in college where the exposure levels are higher. Our previous research work also showed that 7 of the 13 voice students exceeded 100% noise dose in one of the two measurement days. Thus, it can be expected that voice student musicians are exposed to more intense sounds than non-music students. The high sound level exposure in voice students might cause more neuronal damage than non-student musicians which might be evident in the form of reduced ABR wave I amplitude.

Hypothesis 3: The amplitude of ABR wave I will be lower in student musicians with (higher exposure level) brass instruments than student musicians of voice (low exposure level).

Rationale: The sound levels produced by different music instruments vary widely. Phillips and Mace (2008) measured sound exposure levels in practice rooms of student musicians with different primary instruments and reported that for brass players, mean

sound level was 95.2 dBA (for mean measured period of 38.4 minutes) while for vocalists, the mean sound level was 88.4 dBA (for mean measured period of 39.3 minutes). Furthermore, in band settings like orchestra, musicians who play principal trumpet, horn, and trombone in orchestra are at highest risk of exposure to excessive sustained noise levels (Washnik, Phillips, and Teglas, 2016). As evident from these studies brass players exposed to higher sound levels during individual practice and group performances compared to other student musicians, including vocalists. The higher sound exposure in brass players might cause more neural loss than voice students which would consequentially result in a lowering of wave I amplitude.

CHAPTER III

METHOD

The purpose of the present study was to determine the difference in auditory nerve functioning between student musicians and non- music students and between brass players and vocalist as a function of reported noise exposure background. The noise exposure background among participants was determined using a questionnaire. The rationale for inclusion of student musicians in the present study was that student musicians are more frequently exposed to hazardous sound levels than non-student musicians. The higher noise exposure in student musicians may induce permanent neural damage without hair cell loss which could be manifested in the form of reduce amplitude of ABR wave I at suprathreshold levels. Furthermore, within student musicians there is some variability in terms of sound exposure levels due to different primary instruments. Students who play brass instruments are exposed to higher sound levels than vocalists (Phillips and Mace, 2008; Washnik et al, 2016). It could be expected that student musicians who play a brass instrument may show reduced amplitude of ABR wave I compare to vocalists.

Participants:

Student musicians and non-music major students within the age range of 18-30 years were invited to participate in the study. A total of 75 students were recruited from University of North Carolina at Greensboro (UNCG) which were sufficient to provide 0.8

power and 0.8 effect size for pairwise comparisons. 25 student musicians playing brass instruments (i.e. trumpet, trombone, tuba, horn, saxophone, and euphonium) and 25 singers/vocalists (voice major students) were recruited. 25 Non-music major students were recruited from different departments in the UNCG campus. Students were informed about the ongoing research project through flyers. Multiple invitation emails were sent to brass students, voice students, and non-music major students and participants were recruited on a first come first serve basis.

Inclusion Criteria:

1. Pure tone hearing thresholds at frequencies 1, 2, 3, 4, 6, and 8 kHz should be ≤ 15dBHL.
2. Normal otoscopic examination.
3. Normal middle ear function shown on immittance measurements with type A tympanogram (pressure ranging from +100 to -100 dapa, compliance within 0.33 to 1.75 cc, and ear canal volume between 0.8 to 1.8 cm^3).
4. Acoustic reflexes must be present on at least 2 out of 3 frequencies (500 Hz, 1000 Hz, and 2000 Hz).
5. Ethnicity: European descent. African Americans were not included as the previous research suggest that they are more resistant to NIHL than populations of European descent (Jerger, Jerger, Pepe, and Miller, 1986).

Exclusion Criteria:

1. Participant with threshold exceeding 15 dB HL at any frequencies (1, 2, 3, 4, 6, and 8 kHz) were excluded.
2. Participants with history or complaint of tinnitus, head trauma, neurological disorder, active external or middle ear disorder were excluded from the study.
3. Participants with abnormal findings on immittance measurements were excluded.

Data Collection Procedures:

The current study was approved by the Institution Review Board, UNCG. Students from UNCG campus were informed about the ongoing research project through flyers. Multiple invitation emails were sent to brass students, voice students, and non-music major students and participants were recruited on a first come first serve basis. Participants who responded positively to the invitation emails were asked to fill up the online noise exposure screening questionnaire at least 1 week before reporting to the clinic for testing.

Based on the responses on the online noise exposure questionnaire, participants were shortlisted and appointments for testing were scheduled through email. All participants were informed through email that they should refrain from loud sound exposure 12 hours before reporting to the audiology clinic for testing.Participant reported to the audiology clinic on the scheduled time at Ferguson building in UNCG campus. After reporting to the audiology clinic, participants were asked to complete an informed consent form followed by a brief case history. The researcher took a short case history

which comprised of questions pertaining to health issues such as neurological disorders, tinnitus, middle ear disorders, epilepsy, and head trauma.

Thereafter, participant's external ear canal was examined through an otoscope followed by a screening immittance audiometry and reflexometry to rule out any middle ear pathology. The immitance test and reflexometry was performed using diagnostic GSI Tympstar instrument for both ears. After immittance and reflexometry tests, pure tone screening audiometry was carried out using AC-40 diagnostic audiometer and insert earphones. Hearing thresholds of each participant was obtained at 1000, 2000, 3000, 4000, 6000, and 8000 Hz for both ears, using the modified Hughson-Westlake procedure.

After the completion of screening audiometry, suprathreshold auditory brainstem response test was administered using IHS smartEP instrument at audiology clinic in Ferguson building. For ABR test, every participant was asked to recline on a comfortable chair. Participants were allowed to sleep or remain awake during the ABR test. A two channel suprathreshold ABR recording was conducted on left ear of all participants at three stimulus levels i.e. 90, 75, and 60 dB nHL using clicks stimuli. The left ear was chosen for ABR test because left ear is more frequently affected than right ear in NIHL (McBride & Williams, 2001; Nageris et al, 2007; Phillips et al, 2008).

Noise Exposure Screening Questionnaire:

The purpose of administering a noise exposure screening questionnaire in this study was to establish a level of uniformity among three groups (i.e. brass players, vocalists, and non-music students) in terms of noise exposures other than music. This questionnaire has two parts, the first part deals different sound exposure other than

exposure through musical instruments in the last one year. The second part of this questionnaire is related to exposure through musical instruments. The first part of the questionnaire was divided into 9 sections which deals with different types of exposure like noise exposure through firearms, crackers, power tools, heavy equipment, aircraft, music through head phones and speakers, and operated motorized vehicles. The second part of the questionnaire has 9 questions specific to musical instrument played by the participants.

Exclusion criteria based on the responses obtained through noise exposure screening questionnaire:

- Any participant who reported participation in firearm shooting session or hunting on monthly, weekly, or daily basis was excluded. The rationale behind excluding such participants is previous literature which suggest that firearms might cause acoustic trauma (Ylikoski, 1987, 1989).
- Any participant who reported daily or weekly exposure to power tools, heavy equipment, aircraft, and motorized vehicles will be excluded. The daily or weekly noise exposure of such kind might cause subtle neuronal damage and thus, this damage could not be attributed to exposure through music instrument.
- Any participant reporting exposure to power tools, heavy equipment, air craft, and motorized vehicles within the period of last month was not included in the study.

- Participants who reported listening to music through head phone or speakers more than 6 hours on daily basis were excluded.
- Any non-musician participant who reported minor in music or history of playing any instrument or singing in small or large bands was not included in the non-musician group.

Auditory Brainstem Response (ABR) Assessment Protocol:

The suprathreshold ABR responses of each participant was obtained using a commercial system (Smart EP- Intelligent hearing system, Miami, FL) in a single-walled room. Stimulus level calibration in dB nHL was attained by measuring behavioral thresholds to each stimulus in 10 normal hearing adults. The following recording and stimuli parameters will be used to evoke the ABR;

- A two-channel setting was used for obtaining ipsilateral ABR responses with tiptrode and mastoid electrode simultaneously from left ear. The channel 1 inverting electrode was placed on mastoid of left ear while channel two inverting electrode (i.e. tiptrode) was placed on left ear canal. The 2 non-inverting electrodes of two channels were unified into a single non-inverting electrode by using a jumper. The non-inverting electrode was placed on participant's vertex (Cz), whereas the ground electrode was placed on low forehead. A gold foil tiptrode electrode is closer to neurological site of wave I than earlobe electrode. Thus, we expected that wave I with a tiptrode will be more enhanced and clear than wave I with mastoid electrode. The study by Stamper and Johnson (2015) did not reveal significant association between

noise exposures and wave I amplitude due to higher variability in wave I amplitudes using tympanic membrane electrodes. We expect that this variability in wave I amplitude will be less with gold foil tiptrode.

- An 8 ms time window will be used with a filter setting of 30-1500 Hz.
- Two repetitions of 2000 sweeps were accomplish at 90, 75 and 60 dBnHL. It was decided limit surthreshold stimulus level till 60 dB nHL because the previous study by Stamper and Johnson (2015) did not find any significant association below 60 dB nHL.
- Stimuli: Clicks (100 μsec) were presented using insert earphones. The rationale for using clicks was that it is a broadband signal with a range from 3000 Hz to 6000 Hz. This frequency range is typically affected due to excessive noise exposure.
- Stimuli were presented at stimulus rate 11.1/sec with rarefaction polarity in order to enhance wave I (Ruth, Hildebrand, and Cantrell, 1982).
- Stimulus presentation level begun at 90 dB nHL and decreased in 15 dB steps till 60dB nHL.

After the termination of ABR testing of each participant, the two replications at each intensity levels were averaged and the averaged waveform was used for analyzing amplitudes and latencies. Amplitude of ABR wave I was calculated from the difference in voltage at the positive peak and the voltage at the following negative dip. The averaged ABR waveforms were analyzed by two audiologists, Nilesh Washnik and Dr. Denise Tucker. Both audiologists reviewed the data together and were in agreement for

all the marked peaks of waves and their amplitude. This procedure was completed separately for waveforms obtained with a tympanic membrane and a mastoid recording site.

Data Analysis and Statistical Plan:

The suprathreshold ABR wave I data was a nested data at three levels (i.e. at the level of groups, placement, and intensity). The amplitude data was nested within the intensity levels, the intensity levels were nested within electrode placement levels, and the electrode placements were nested within the three groups (i.e. Non-musicians, brass majors, and voice majors group). For this nested data, Repeated Measure ANOVA was utilized to analyze the main effects of the intensity, placement, intensity level, Pure tone average thresholds (PTA 234 kHz), and the interaction between groups, placements, intensity levels, and PTA 234 kHz. The main effect for the groups was significant, whereas no significant interaction was found between groups and other variables. Tukey's test was used to examine all possible pairwise comparisons between the groups. The rationale behind opting to use Tukey's test was to control the type I error rate.

The main effect for interaction between intensity and placement was significant. In order to control family wise Type I error rate and examine all possible pairwise comparisons between different levels of intensity and placement settings, Tukey's test was used. All necessary statistics procedure was completed using SAS Enterprise guide, version 7.13, Cary, NC, USA.

CHAPTER IV

RESULTS

The aim of the study was to identify differences in auditory nerve functioning between collegiate non-music students and music major students. Suprathreshold ABR wave I amplitude was used to compare the auditory nerve functioning between the non-musician students group (group 1), brass major students group (group 2), and voice major students group (group 3).

Descriptive statistics for gender and age are shown in Tables 1 and 2 for all three groups. There was no significant difference in age between the groups. Figure 1 shows the range and median values of PTA234 kHz of all the three groups, which are no more than 3 db apart and are clinically insignificant.

Table 1. Descriptive Statistics of Gender

	Groups			Total
	Nonmusicians	Brassmajors	Voicemajors	
Female	23	8	21	52
Male	2	17	4	23
Total	25	25	25	75

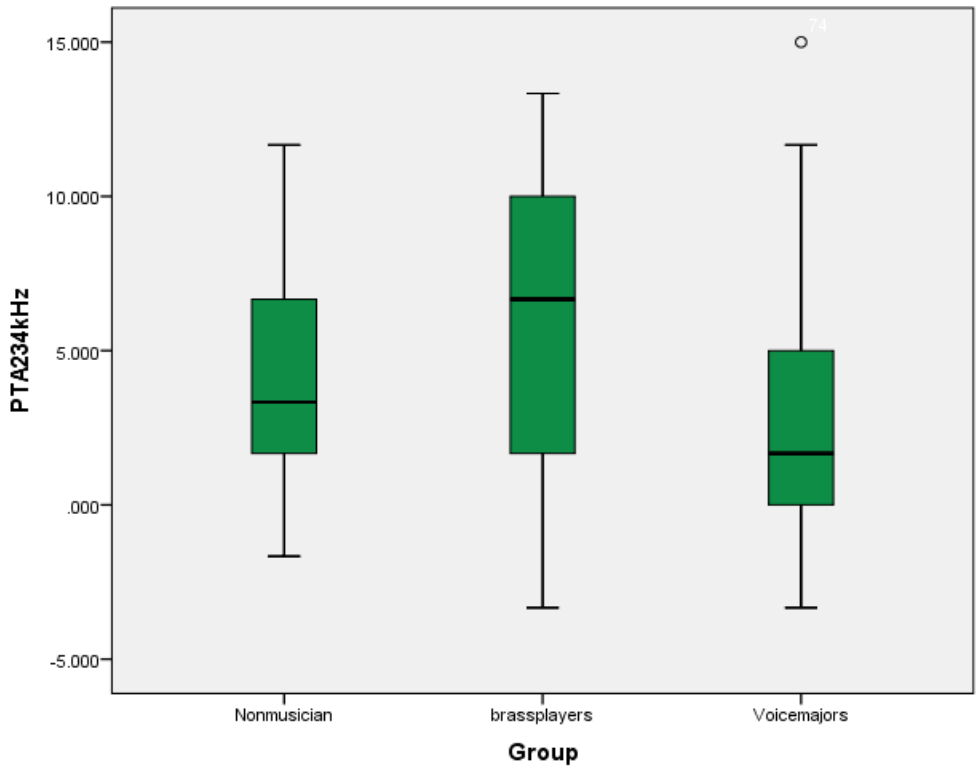

Figure 1. Pure Tone Average of Thresholds at 2, 3, and 4 kHz (PTA234kHz) in Non-Musicians, Brass Majors, and Voice Majors Group

Suprathreshold ABR Data:

The suprathreshold ABR wave I data is a nested data at three levels (i.e. at the level of groups, placement, and intensity). The amplitude data was nested within the intensity levels, the intensity levels were nested within electrode placement levels, and the electrode placements were nested within the three groups (i.e. Non-musicians, brass majors, and voice majors group). For this nested data, Repeated Measure ANOVA was utilized to study the main effect between the groups and the interaction of wave I amplitude at the above mentioned three levels. The main effect of groups, intensity, placement and the interaction is shown in Appendix B.

There was no statistically significant interaction between group and intensity (p=0.1948) and between group and placement (p=0.0651). Thus, we studied the main effects of groups without subdividing it at placement and intensity levels.

Results of the Hypotheses Testing:

Hypothesis 1: Student musicians with brass instruments will exhibit a reduced amplitude of wave I ABR at suprathreshold levels when compared with non-music major students.

Tukey's test was used for group comparisons. The rationale for using Tukey's test was to control for a type I error while comparing the three groups. Table 2 shows the results of Tukey's test. There was a statistically significant difference between the non-musicians group and brass majors group (Adjusted p =0.0095). The results indicate that the participants of the brass majors group had a statistically significant reduced wave I amplitude compared to participants from the non-musicians group.

Hypothesis 2: The amplitude of ABR wave I will be lower in student musicians of voice than non-music students.

A statistical significant difference was observed between the non-musicians and voice majors groups for wave I amplitude (Adjusted p=0.0428). The results of the test indicate that the voice major group had a statistically significant lower wave I amplitude than non-musicians group. The wave I amplitude is significantly higher in non-musicians group compare to voice majors group.

Table 2. Summary of Tukey's Test: Comparisons between Non-Musicians, Brass Majors, and Voice Majors Group

Group	Group	Estimate	Std. Error	DF	t value	Pr> /t/	Adjusted P
Non-musicians	Brass majors	86.8667	28.7019	72	3.03	0.0034	**0.0095**
Non-musicians	Voice majors	70.5333	28.7019	72	2.46	0.0164	**0.0428**
Brass majors	Voice majors	-16.3333	28.7019	72	-0.57	0.5711	0.8370

Hypothesis 3: The amplitude of ABR wave I will be lower in student musicians with (higher exposure level) brass instruments than student musicians of voice (low exposure level).

The results of Tukey's test, as seen in Table 2, reveals no statistically significant difference between the brass majors and voice majors groups (Adjusted $p = 0.8370$), indicating that the amplitude of wave I for brass majors and voice majors group was not significantly different.

Wave I Amplitude: Interaction between Groups, Placement, and Intensity Levels:

To analyze the interaction of wave I amplitude between groups, placement settings, and intensity levels, a Repeated Measure ANOVA was done. Appendix B shows the main effect of interaction of all these independent variables. The main effects for group-intensity interaction (p=0.1948), group-placement interaction (p=0.0651), and group-intensity-placement interaction (p=0.9645) were not significant. The main effect for intensity-placement interaction was statistically significant ($p<0.0001$, Appendix B). Tukey-Kramer's test was used to study the intensity-placement interaction at different intensity and placement combinations. Appendix C reveals the result of Tukey-Kramer test for all possible pair-wise interactions between different intensity levels and placement. Statically significant difference was found at all possible intensity-placement pairwise interactions.

Influence of Electrode Placement on Wave I Amplitude:

Table 3 shows the amplitude of the ABR wave I in the current study. Means and Standard Deviation of wave I are provided for click evoked ABR responses obtained at 90, 75, and 60 dB nHL with Tiptrode (TT) and Mastoid (MT) electrode placement settings. The mean amplitude values are highest in the non-musician group with both electrode setting at all intensity levels.

In Figures 2 and 3, the marginal mean amplitudes of wave I obtained at different intensity levels (i.e. 90, 75, and 60 dB nHL) with mastoid and tiptrode for non-musicians (blue line) are significantly larger than marginal mean amplitudes of wave I with a

mastoid and tiptrode electrode of brass students (red line) and voice students (green line) groups. Comparison of these two figures show the potential of tiptrode to increase the wave I amplitude because tiptrode is closer to the generating site of wave I than mastoid electrode.

Table 3. Summary of Mean and SD of Wave I Amplitude of Three Groups at 90, 75, and 60 dB nHL Obtained with Mastoid (MT) and Tiptrode (TT) Placement

			Non-musician		Voice majors		Brass Major	
Intensity	Placement	n	Mean	SD	Mean	SD	Mean	SD
90 dB nHL	TT	25	714.80	223.87	588.00	216.35	616.40	198.09
	MT	25	418.40	150.38	356.00	160.07	335.20	111.24
75 dB nHL	TT	25	610.00	181.68	503.20	170.92	500.40	183.50
	MT	25	356.80	108.39	290.00	116.97	256	92.96
60 dB nHL	TT	25	185.20	91.24	152.00	99.07	115.60	71.60
	MT	25	117.20	64.71	111.20	81.30	60.40	42.07

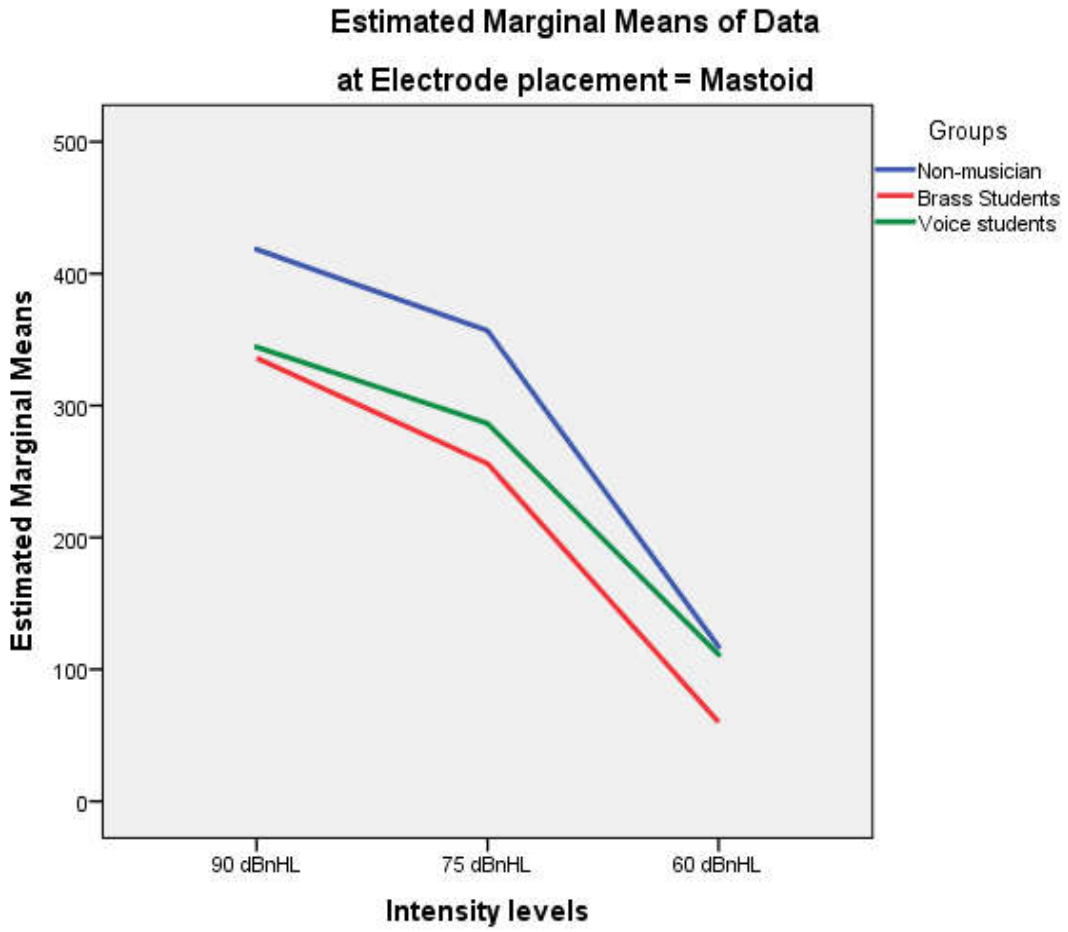

Figure 2. Estimated Marginal Means of ABR Wave I Amplitude at 90, 75, and 60 dB nHL with Mastoid Electrode Placement of all the Three Groups

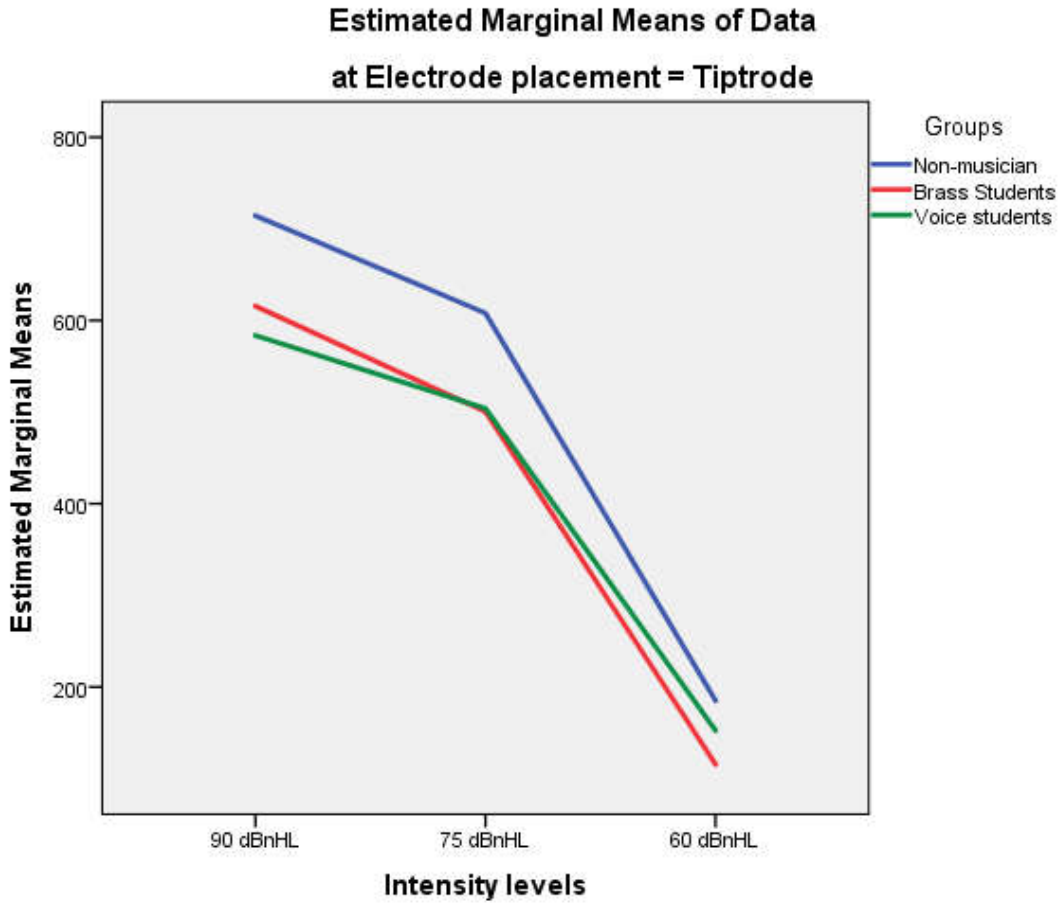

Figure 3. Estimated Marginal Means of ABR Wave I Amplitude at 90, 75, and 60 dB nHL with Tiptrode Electrode Placement of all the Three Groups

Influence of Pure Tone Average of 2, 3, and 4 kHz on Wave I Amplitude:

All the participants recruited in this study had normal hearing. The normal hearing in this study was defined as behavioral thresholds at 0.5, 1,2,3,4,6, and 8 kHz ≤ dBHL. This definition of normal hearing encompasses 25 dB range (i.e. -10 dBHL to 15 dBHL). The variation in behavioral thresholds within this 25 dB range has the potential to affect the amplitude of ABR wave I. To assess the association between behavioral thresholds and ABR wave I amplitude among groups, a Repeated Measure ANOVA was completed.

As the stimuli used in the ABR test was clicks, which is a high frequency broadband signal, the pure tone average of 2, 3, and 4 kHz was used as a substitute for click threshold.

The results of the Repeated Measure ANOVA are shown in Appendix B. The results indicated that there was no statistically significant association between behavioral thresholds and wave I amplitude for ABR responses of the three groups. In summary, the outcome of the analysis suggests that the behavioral threshold did not affect the ABR wave I amplitude and the variation in hearing thresholds was not a confounding factor in this study.

CHAPTER V

DISCUSSION

The aim of this research study was to identify the difference in auditory nerve functioning between collegiate non-music students and music major students. Three hypothesis were presented: 1) Student musicians with brass instruments will exhibit a reduced amplitude of ABR wave I at suprathreshold levels when compared with non-music major students, 2) The amplitude of ABR wave I will be lower in student musicians of voice than non-music students, 3) The amplitude of ABR wave I will be lower in student musicians with (high exposure level) brass instruments than student musicians of voice (low exposure level). These hypotheses will be discussed one by one, along with other findings.

Hypotheses 1 and 2:

The two major findings of the present study are; 1) Student musicians with brass instrument showed significantly reduced ABR wave I amplitude (Adjusted p=0.0095), compared to the non-musician students, 2) The amplitude of ABR wave I was significantly reduced in voice major students (Adjusted p=0.0428) compared to non-musician students. There was no statistically significant interaction between group and intensity (p=0.1948) and between group and placement (p=0.0651).

These significant differences in wave I amplitude between non-music studentsand brass major student groups and between voice major and non-musician groups are likely to be attributed to the difference in noise exposure background. Participants from all the groups met the criteria of noise exposure screening questionnaire. None of the participants from non-music students group reported playing any musical instrument or practicing music in the noise exposure screening questionnaire.

The two major findings of the present study are consistent with the findings from the animal studies where smaller wave I amplitudes were seen at suprathreshold levels in noise exposed animal ears than controlled animal's ears (Kujawa and Liberman, 2009; Lin et al., 2011; Wang and Ren, 2012; Furman et al. 2013; Fernandez et al, 2015; Jensen, Lysaght, Liberman, Qvortrup, & Stankovic, 2015) and findings from the human studies (Stamper And Johnson, 2015; Bramhall, Konrad-Martin, Mcmillan, & Griest, 2017; Prendergat et al., 2017).

The results of previous investigations suggested that loud sound exposure through music practice in student musicians might cause similar damage of auditory nerve fibers and synaptic ribbons of IHCs as found in animal studies by Kujawa and colleagues. The results of the present study suggest that the reduced amplitude of ABR wave I in brass major and voice major groups compared to the non-musician group may be a consequence of damage to nerve fibers innervating IHCs from the modiolar side. These nerve fibers, with low spontaneous rates and high thresholds, are crucial for our ability to detect a signal in the presence of noise because they are more resistant to masking (Costalupes et al., 1984). Thus, it can be expected that musicians' ability to detect

signal/speech in presence of noise might be compromised due to damaged auditory nerve fibers with high threshold and low spontaneous rate. However, it has been well documented that musicians excel in their ability to detect signal in presence of noise compared to non-musicians (Parbery-Clark, Skoe, & Kraus, 2009; Parbery-Clark, Skoe, Lam, & Kraus, 2009; Parbery-Clark, Strait, Anderson, Hittner, & Kraus, 2011).

Hearing signal/s in noise is dependent on the ability to differentiate and track relevant signals from the background noise, and recognizing the unique timbral signature of the target signal is a right way to achieve this. Perception of timbre is driven by both envelope and harmonic encoding (Krimphoff, McAdams, & Winsberg, 1994) and both of these components play an important role in hearing in noise. Student musicians have more robust representation of envelope, stimulus-to-response and harmonic encoding than non-musicians. The strengthened encoding of these spectral features may provide musicians the ability to better recognize and segregate signals, giving them an advantage for enhanced speech perception in noise. (Parbery-Clark et al., 2009, 2011; Zendel and Alain, 2011; Strait et al., 2011).

Musicians have extensive pervasive subcortical specialization including the brainstem that improves auditory encoding of music and speech in noise (Musachia, Sams, Skoe, & Kraus, 2007). This subcortical specialization in musicians might compensate for the peripheral damage of nerve fibers with high threshold and lower spontaneous rate. Thus, it is possible that despite damaged auditory nerve fibers with high and low spontaneous rate the ability of student musicians to detect signals in the presence of noise remains unaffected due to music-induced subcortical modifications.

The biological mechanism underpinning this subcortical specialization remains a topic of debate. The two possible mechanism are as follows, (a) local neuronal reorganization occurring within the brainstem (b) top-down modulation through descending neuronal tracts is driven by enhanced higher-level control over basic sensory processing. (Krishnan & Gandoura, 2009).

In the present study, participants were informed to refrain from loud sound exposure for at least 12 before reporting to the audiology for ABR test. It is quite possible that there might be some student musicians who might have developed TTS due to loud sound exposure which occurred within the last 24 hours. Complete recovery from TTS may take a few minutes to a few weeks (Ward, 1970; Kujawa & Liberman, 2009). Thus, there might be some student musicians who did not recover from TTS completely and this incomplete recovery might have influenced the ABR wave amplitude. However, all of the participants showed normal hearing and the interaction between the average hearing thresholds and wave I amplitudes among the three groups was not significant. Thus, the possibility of incomplete recovery from TTS among student musicians was not likely to be a confounding factor.

One of the important similarities in all of the above-mentioned studies showing reduced ABR wave I amplitude as a function of noise exposure and the present study is that the hearing thresholds of participants were within normal limits. Threshold responses rely on the synchronous firing of auditory nerve fibers, but are contingent upon a criterion response only slightly above the noise floor. Thus, normal hearing threshold

responses are relatively unaffected by significant changes in auditory nerve fiber populations (Schuknecht and Woellner, 1953; Earl and Chertoff, 2010).

Hypothesis 3:

There was no statistically significant difference in wave I amplitude between student musicians with brass instruments and student musicians of voice (Adjusted p= 0.8373). The estimated marginal means values of wave I amplitude in the brass major group (312.87 nV) was lower than the voice major group (329.20 nV) but this difference was not significant. This finding could be attributed to the fact that student musicians from both the groups were exposed to loud music on a daily basis. Washnik et al. (2016) measured noise doses in collegiate student musicians and found that 11 out of 11 brass students exceeded 100% daily noise dose on at least one of two measurement days, whereas 7 out of 13 voice major students exceeded 100% daily noise dose on at least one of two measurement days. Although the vocalists were exposed to lower sound exposure levels than brass major students, the lack of significant difference between brass major student and voice major students in the present study suggest that the lower exposures of the vocalists is adequate enough to damage the auditory nerve fibers. The regular and voluntary high level music exposure in both groups might cause similar damage of auditory nerve fibers and synaptic ribbons of IHC which ultimately result in similar reduction of wave I amplitude in brass major and voice major groups.

Influence of Intensity and Placement of Electrode:

The interaction between intensity and placement was the only statistically significant interaction ($p<0.0001$). For all possible combinations of intensity

levels and electrode placement settings, a significant interaction was found (Appendix III). These results reveal that at the same intensity levels, the wave I amplitude obtained with tiptrode placement was significantly higher than mastoid placement irrespective of group. This result was expected, as the tiptrode is closer to the generating site of wave I than the mastoid electrode (Bauch and Olsen, 1990). Stamper and Johnson (2015) found a similar pattern in their study; the only difference was that they used a tympanic membrane electrode instead of a tiptrode, and found a higher amplitude with the tympanic membrane electrode than the mastoid electrode at suprathreshold levels.

Clinical Applications:

The outcomes of this study reveals that the normal hearing student musicians with brass instruments and voice exhibit reduced ABR wave I amplitude compare to non-musician students. This reduced ABR wave I amplitude is an indication of damaged auditory nerve fibers with high threshold and low spontaneous rate. Student brass and voice musicians (vocalists) are frequently exposed to high sound levels through extended practicing and performing in ensembles. Such exposure might result in permanent damage of specific auditory nerve fibers without affecting hearing thresholds which is manifested in the form of reduced amplitude of ABR wave I.

The results of the current study indicate that ABR wave I amplitude is a sensitive tool to identify subtle nerve fiber damage occurring in population at risk for NIHL. The ABR wave I amplitude could be used in assessing the susceptibility to develop NIHL in humans who are frequently exposed to hazardous sound levels. Click evoked ABR responses obtained with either mastoid or tiptrode mastoid electrode at optimum

suprathreshold levels like 80 or 75 dB nHL was adequate to identify differences in wave I amplitude between student musicians groups and the non-music student group. In order to include ABR wave I in clinical protocols for NIHL additional research needs to be conducted on normal hearing populations with history of significant noise exposure for developing normative value and standard methods to measure the amplitude of ABR wave I.

Limitations and Future Implications:

Every research study has its own set of limitations thus providing for further exploration. A few limitations and suggestions are mentioned below.

1. Future research is needed to develop normative values of ABR wave I amplitude at suprathreshold levels which could be used in clinical protocols as an indicator of early signs of NIHL.
2. The neural damage induced due to excessive noise exposure might impact the amplitude or latency of the ipsilateral reflexes. Future research studies could analyze the latency and amplitude of ipsilateral reflex as a function of noise exposure history.
3. Gender is an important factor which was not considered during data collection. Future research could control this factor and observe its effects.
4. In the current study, only participants with European descent were included. African Americans were not included as previous research suggests that they are more resistant to NIHL than populations of European descent (Jerger, Jerger, Pepe, and Miller, 1986; Lin, Thorpe, Gordon-Salant, Ferrucci, 2011).

A similar kind of study can be conducted on African-American or other races to confirm that this phenomenon is independent of skin color.

5. Number of years of practicing music among student musicians was not considered during data collection. Future studies can analyze the effect of numbers of years of practicing music on ABR wave I amplitude.
6. There is a need to identify sound exposure levels causing TTS without reducing the ABR wave I amplitude. Identification of such levels will help in modifying damage risk criteria for NIHL.

Conclusion:

The results of this study reveal that the normal hearing student musicians with brass instruments and voice exhibit reduced ABR wave I amplitude compare to non-musician students. This reduced ABR wave I amplitude is suggestive of damaged auditory nerve fibers with high threshold and low spontaneous rate. These fibers are crucial for detecting signal in presence of noise because they are resistant to masking. Ironically it is well documented fact that musicians outperformed non-musicians in tasks pertaining to perception of signals in presence noise. Intensive musical training, enhances subcortical and cortical structures underlying the neural encoding that are crucial for hearing in noise. These modifications in subcortical structures due to musical training might compensate for the peripheral damage of nerve fibers with high threshold and lower spontaneous rate. The biological mechanism which cause this subcortical modification is unclear and needs to be investigated.

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APPENDIX A

NOISE EXPOSURE SCREENING QUESTIONNAIRE

Name (First name & last name): _____

Email id: _____ Age/Sex: _____

Please indicate your predominate race ancestry
□ Native American □ African American □Caucasian □ Asian □ Polynesian □ Other

Do you play any musical instrument? □ Yes □ No

Are you a music major? □ Yes □ No

Name of the instrument you play _____

Part I

In the last 1 year

1. How often have you shot firearms such as rifles, pistols, shotguns, etc.?
□ Never □Every few months □ Monthly □ Weekly □ Daily

2. If you were around/shot firearms, on average, how many shots did you fire each time/session?
_____ **shotgun/rifle** shots per session _____ **pistol** shots per session

3. How often have you been exposed to fireworks or other fire crackers?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

4. If you were around fireworks, on average, how many fireworks did you shoot each time/session?
_____ firecracker/firework shots per session

5. How often do you listen to music through earphones/ headphones/earbuds?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

6. When you listen music through speakers or head phone, how many hours does each session last/
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

7. How often do you listen to music through speakers in a car or at home?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

8. When you listen music through speakers, how many hours does each session last/
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

9. How often have you attended car/truck races, commercial/high school sporting events, music concerts/dances or any other events with amplified public announcement (PA)/music systems?
□Never □ Every few months □ Monthly □ Weekly □ Daily

10. If you attended these events, on average, how many hours did each time/session last?
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

11. How often have you used power tools, chainsaws, or other shop tools?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

12. When was the last time you exposed
□ Within the last week □within the last two weeks □ within the last month

13. If you used power tools, on average, how many hours did each time/session last?
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

14. How often have you driven heavy equipment or use loud machinery (such as tractors, trucks, or farming or lawn equipment like mowers/leaf blowers)?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

15. When was the last time you exposed
□ Within the last week □within the last two weeks □ within the last month

16. If you drove/used loud machinery, on average, how many hours did each time/session last?
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

17. How often have you ridden/operated motorized vehicles (excluding cars) such as, jet skis, speed boats, snowmobiles, etc.?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

18. When was the last time you exposed
□ Within the last week □within the last two weeks □ within the last month

19. If you rode motorized vehicles, on average, how many hours did each time/session last?
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

20. How often have you ridden in or piloted small aircraft/private airplanes?
□ Never □ Every few months □ Monthly □ Weekly □ Daily

21. When was the last time you exposed
□ Within the last week □within the last two weeks □ within the last month

22. If you flew airplanes, on average, how many hours did each time/session last?
□ 6 to 8 hours □ 3 to 6 hours □ 1 to 3 hours □ less than an hour

Part 2

This segment of questionnaire is for student musicians or those students who play at least one musical instrument on a regular basis.

Musical Instrument

Primary instrument: _____

1. How often did you play a musical instrument?
   □ Never □ Every few months □ Monthly □ Weekly □ Daily

2. If you played a musical instrument, on average, how many hours did each time/session last?
   □ 4-6 hours □ 3 hours □ 2 hour □ 1 hour □ less than1 hour

3. On average, how many hours do you practice with your primary instrument (including individual practice and ensemble rehearsals) in a week? _____

4. **Secondary Instrument: _____**

5. How often do you play your secondary instrument?
   □ Never □ Every few months □ Monthly □ Weekly □ Daily

6. If you played a secondary musical instrument, on average, how many hours did each time/session last?
   □ 4-6 hours □ 3 hours □ 2 hour □ 1 hour □ less than1 hour

7. On average, how many hours do you practice with your secondary instrument (including individual practice and ensemble rehearsals) in a week? _____

8. How often do you observe other student's practice or performance?
   □ Never □ Every few months □ Monthly □ Weekly □ Daily

9. If you observe other student's or band's performance, on average, how many hours did each time session last?
   □ 4-6 hours □ 3 hours □ 2 hours □ 1 hour □ less than1 hour

APPENDIX B

SUMMARY OF REPEATED MEASURE ANOVA ANALYSIS: MAIN EFFECTS FOR GROUPS, INTENSITY, PLACEMENT, GROUP-PLACEMENT INTERACTION, GROUP-INTENSITY INTERACTION, INTENSITY-PLACEMENT INTERACTION, AND GROUP-INTENSITY-PLACEMENT INTERACTION

Effect	Num DF	Den DF	F value	Pr>F
Groups	2	72	5.17	0.0080
Intensity	2	144	339.66	<.0001
Placement	1	216	677.58	<.0001
Groups*Intensity	4	144	1.54	0.1948
Groups*Placement	2	216	2.77	0.0651
Intensity*Placement	2	216	87.94	**<.0001**
Groups*Intensity*Placement	4	216	0.15	0.9645

APPENDIX C

SUMMARY OF TUKEY-KRAMER TEST FOR INTENSITY PLACEMENT INTERACTION

Effect	**Inty (dB)**	**Plmt**	**Inty (dB)**	**Plmt**	**Estmt**	**t Value**	**Pr > |t|**	**Adj P**
Intensity*Placement	60	1	60	2	-54.00	-4.35	<.0001	**0.0003**
Intensity*Placement	60	1	75	1	-203.33	-11.57	<.0001	**<.0001**
Intensity*Placement	60	1	75	2	-436.80	-24.84	<.0001	**<.0001**
Intensity*Placement	60	1	90	1	-269.47	-15.33	<.0001	**<.0001**
Intensity*Placement	60	1	90	2	-541.60	-30.81	<.0001	**<.0001**
Intensity*Placement	60	2	75	1	-149.33	-8.49	<.0001	**<.0001**
Intensity*Placement	60	2	75	2	-382.80	-21.77	<.0001	**<.0001**
Intensity*Placement	60	2	90	1	-215.47	-12.26	<.0001	**<.0001**
Intensity*Placement	60	2	90	2	-487.60	-27.73	<.0001	**<.0001**
Intensity*Placement	75	1	75	2	-233.47	-18.81	<.0001	**<.0001**
Intensity*Placement	75	1	90	1	-66.1333	-3.76	0.0002	**0.0029**
Intensity*Placement	75	1	90	2	-338.27	-19.24	<.0001	**<.0001**
Intensity*Placement	75	2	90	1	167.33	9.52	<.0001	**<.0001**

Intensity*Placement	75	2	90	2	-104.80	-5.96	<.0001	**<.0001**
Intensity*Placement	90	1	90	2	-272.13	-21.93	<.0001	**<.0001**

*Inty=Intensity; Plmt= Electrode Placement, 1=mastoid placement, 2= tiptrode placement