EFFECTS OF AGEING AND NOISE EXPOSURE ON ABR AND DPOAEs

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CERTIFICATE

This is to certify that this dissertation entitled '**Effects of ageing and noise exposure on ABR and DPOAEs'** is a bonafide work submitted in part fulfilment for degree of Master of Science (Audiology) of the student Registration Number: 16AUD015. This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru
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CERTIFICATE

This is to certify that this dissertation entitled '**Effects of ageing and noise exposure on ABR and DPOAEs'**has been prepared under my supervision and guidance. It is also been certified that this dissertation has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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DECLARATION

This is to certify that this dissertation entitled **'Effects of ageing and noise exposure on ABR and DPOAEs'** is the result of my own study under the guidance a faculty at All India Institute of Speech and Hearing, Mysuru, and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru,
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction

The sounds we hear are of different types and sources, and if not in the safe levels it can lead to damage to the auditory system and cause hearing loss which can vary in degree (Wheeler, 1951). The protocols used clinically for evaluating NIHL relies strongly upon behavioral measures like pure tone audiometry, where the hallmark of NIHL is a high frequency notching seen at 3000 -6000 Hz region. It is accepted widely that the permanent threshold shift (PTS) following noise exposure is caused due to a permanent damage to the auditory structures. An assumption underlying the concept of temporary threshold shift (TTS) is that following full recovery of threshold(s), no residual anatomical damage is present and this decrease in hearing seen temporarily is essentially harmless (Humes, Joellenbeck, & Durch, 2005; Kujawa & Liberman, 2009). Recent animal studies have revealed that exposure to noise causes not just temporary threshold shifts, but can also produce permanent damage to the synapses in the cochlea, termed as 'cochlear synaptopathy'. Cochlear synaptopathy is the loss of synapses and cochlear-nerve terminals innervating inner hair cells (Kujawa & Liberman, 2009; Furman, Kujawa, & Liberman, 2013; Liberman &Liberman, 2015). There are also evidences to show that ageing also has the same mechanism i.e., there is loss of synaptic connections, which is independent of hair cell loss (Schmiedt, Mills, & Boettcher, 1996; Makary, Shin, Kujawa, Liberman, & Merchant, 2011; Sergeyenko, Lall, Liberman,& Kujawa, 2013). When assessed audiometrically in quiet, the thresholds are in normal limits in spite of up to 80% of synaptic loss (Lobarinas, Salvi, & Ding,2013), which makes it difficult to identify using conventional evaluations. Around 5-15% of the adult populations, who come with the complaint of listening in noisy situations or other difficult to hear situations,

have clinically normal audiograms (Hind, Haines-Bazrafshan, Benton, Brassington, Towle,& Moore, 2011).As the problem persists in individuals who have their thresholds within normal range of <20 dB HL, the term 'hidden hearing loss' is coined (Schaette & McAlpine, 2011). The possible cause for such a condition could be many. Findings have shown that both in individuals with noise exposure and in individuals who are aged above 40 years (Elberling &Parbo, 1987; Mitchell, Phillips, & Trune, 1989), there is a permanent destruction of synapses between the inner hair cells (IHCs) and type I auditory nerve fibers (ANFs), thus leading to a slow degeneration of the ANFs. However, the hair cells are not affected leaving the hearing sensitivity to be normal (Kujawa & Liberman, 2015). When administered with Speech-in-Noise test for the clinically normal hearing group (threshold <20 dB HL), there was a high degree of variability in the performance ranging from 82% at an SNR of −1.6 dB in 10% of the best listeners to only 38% at the same SNR in 10% of the worst listeners, suggesting that the use of 20 dB HL as the cut-off is not preferable, especially for the purpose of establishing other clinical norms (Surprenant & Watson, 2001; Gygi, Kidd, & Watson, 2007; Ruggles & Shinn-Cunningham, 2011; Ruggles, Bharadwaj, & Shinn-Cunningham, 2011; Bharadwaj, Masud, Mehrai, Verhulst, & Shinn-Cunningham, 2015).This normative threshold of <20 dB HL is so wide that even modest losses of OHCs are not identified. Previous evidence suggests that these individuals have impaired frequency resolution, but there has been no thorough characterization of auditory filter shapes in this population (Festen & Plomp, 1983; Houtgast & Festen, 2008; Badri, Siegel, & Wright, 2011).

DPOAEs are the sound induced vibrations by the OHCs in the cochlea that are by-products of compressive nonlinear amplification, which enhances both the frequency resolution of hearing and sensitivity (Robles & Ruggero, 2001). The classic

outlook of sensorineural hearing loss (SNHL) is that the hair cells are the primary targets, and deafferentation or cochlear-nerve loss is next to hair cell loss which is the secondary target. However, this outlook towards SNHL has been challenged by many studies recently. One such study was by Kujawa & Liberman (2009) wherein, they induced a temporary NIHL of up to 40dB in guinea pigs and mice; following the recovery from temporary threshold shift (TTS), the auditory system was assessed using distortion product oto-acoustic emissions (DPOAEs) and auditory brainstem responses (ABRs).The results indicated a permanent damage at a frequency region corresponding to maximum TTS in the afferent nerve ending that is between the IHCs and the ANFs. But, there was no significant effect of deafferentation evident in ABR. Therefore, it is been said that the remaining afferent connections that are undamaged would take up the work and help in preserving the threshold within normal range (Kujawa & Liberman, 2009; Lin, Furman, Kujawa, & Liberman, 2011; Furman et al., 2013). Further, a study by Furman et al., (2013) has shown that there is a reduction in the amplitude of wave I after a significant exposure to noise at supra-threshold levels (>40 dBSPL), when the same set of animals were assessed both before and after the noise exposure. It was also reported that there is damage to the low spontaneous rate auditory nerve fibres (low SR ANFs), and concluded that temporary NIHL further leading to hidden hearing loss, affects responses to high levels than at low levels of sound. Therefore, supra-threshold responses of ABR are demonstrated to have better sensitivity in identifying the damage to the auditory structures. Also, the responses to DPOAEs were not affected suggesting normal functioning OHCs (Kujawa & Liberman, 2009; Lin et al., 2011; Furman et al., 2013).

Immunostaining techniques were developed to study age-graded succession of mice to compare synaptic and the hair-cell counts as a measure of cochlear function (ABRs

and OAEs; Sergeyenko et al., 2013).Similar results as obtained by Kujawa and Liberman (2009) were obtained in this study also, that is, the afferent connection between the IHC and the ANF was affected. The synaptic ribbon counts near the IHC reduced monotonically with age but, the hair cell loss was minimal even at a later age (Sergeyenko et al., 2013).

1.1. Need for the study

The view on SNHL in noise exposed and aged individuals have been changed by the fact that there is a hidden component which is neither identified by the audiogram nor the OAEs. So, there is a need to outline an appropriate test to reveal the first signs of these two affected groups. It is a well known fact that after acoustic overexposure, NIHL recovers with an exponential time course (Miller, Watson & Covell, 1963) for 2-3 weeks, depending on initial severity. Studies on animals using invasive techniques reveal that there is no hair cell death in temporary threshold shift; however, swelling of cochlear nerve terminals at their hair-cell synapses is seen within 24 hours of post exposure (Spoendlin, 1971; Liberman &Murloy, 1982). Until recently, based on the animal work, it has been hypothesized that few measures of amplitude at supra-threshold levels in ABR is useful in the non-invasive diagnosis of cochlear synaptopathy. In the same line a non-invasive test to reveal the initial damage in human ears is important to prevent it from further damage as there are very less studies to outline such tests for delineating the changes in auditory system. ABRs are a routinely used test in clinics to provide a non-invasive correlate on hearing sensitivity. Also, ABR responses to transient stimuli are able to locate hearing deficits along the auditory pathway as the abnormalities seen at different waveform peaks stem from aggregate responses of population of neurons at different ascending processing stages (Melcher & Kiang, 1996). Recent studies have reported smaller

ABR wave I amplitudes in noise-exposed animals when compared to the controls that are not exposed to noise (Mehraei et al., 2016). Cochlear synaptopathy predominantly affects suprathreshold processing and has been associated with shallower ABR amplitude versus intensity growth in the presence of normal ABR threshold (Furman et al., 2013). For humans, a significant correlation between high-intensity ABR Wave-I amplitude and noise exposure history was recently reported when the recording was obtained with click stimulus at 90 dB nHL using a mastoid recording electrode. It was reported that ABR wave I amplitudes decreased as a function of noise exposure backgrounds (NEB; Stamper & Johnson, 2015). It is said that low SR ANFs are responsible for perceiving high intensity sounds and these fibers are affected in both individuals with noise exposure and in older age group. Further, a comparison of the effects of synaptopathy at supra-threshold level between the noise exposed and aged individuals is required as it has been said that the same mechanisms are affected in both.

1.2. Aim of the study

The aim of the study is to compare the supra-threshold measures like DPOAEs and ABR in two groups, individuals with noise exposure and aged individuals, with normal hearing.

1.3. Objectives

1. To compare the findings of DPOAEs and ABR in individuals with noise exposure (Group 3) and in aged individuals without noise exposure (Group 2) at various presentation levels (90dB nHL to 50dB nHL, decreasing in 10dB steps).

2. To compare the findings of different tests (DPOAEs and ABR) of the two groups that is, individuals with noise exposure and aged individuals (Group 3 and Group 2) with the normals (Group 1).
3. To find which among the following is a better indicator of ANF loss in both the groups, among amplitudes of wave I and V, latency of wave I and V, and wave V/I ratio.

1.4. Null Hypotheses

The null hypotheses were framed for each of the objectives considered for the study. They were:

1. There is no significant difference in the findings of DPOAEs and ABR in individuals with noise exposure (Group 3) and in aged individuals without noise exposure (Group 2) at various presentation levels (90dB nHL to 50dB nHL, decreasing in 10dB steps).
2. There is no significant difference in the findings of different tests (DPOAEs and ABR) between the two groups that is, individuals with noise exposure and aged individuals (Group 3 and Group 2) with the normals (Group 1).

Chapter 2

Review of Literature

Overexposure to sounds that are intense can cause either temporary or permanent effects on our hearing. However, depending on the severity and duration of the exposure, there is an exponential time-course recovery for over 2-3 weeks (Miller et al., 1963). These thresholds may get elevated and then stabilize in case of permanent threshold shift (PTS) or, it can fully recover in case of temporary threshold shift (TTS) (Liberman & Dodds, 1984). The inner ear consists of two discrete types of sensory cell i.e. the inner hair cells (IHC) that are understood to convey most of the acoustic information to the higher structures through extensive (over 90%) type I synaptic connections whereas, the outer hair cells(OHC) that are responsible for the remaining 10% are said to have type II connections. Hearing impairment as a consequence of ageing, noise exposure, or ototoxicity, classically begins with OHC dysfunction, which is followed by progressive loss of IHC and the spiral ganglion cells (Harding, Bohne, &Vos, 2005; McFadden, Ohlemiller, Ding, Shero & Salvi, 2001; Stebbins, Hawkins, Johnsson, & Moody, 1979). In contrast to the effects of OHC loss, usually seen as an increment in the thresholds at frequencies allied with OHC damage along with failure of frequency selectivity, there are a few models that describe the effects of selective IHC loss on hearing. The intrinsic damage to the IHCs with no significant OHC damage was first understood by a model of selective IHC damage due to ototoxicity developed in chinchillas using moderate to high doses of the anticancer drug, carboplatin (Takeno, Harrison, Mount, Wake, & Harada, 1994). There was a selective loss of IHC (>50%) as an effect of ototoxicity but spared OHCs which was evident as there was no effect on DPOAEs as well as cochlear microphonics (CM), which are considered as the functional measures of OHC

integrity (Hofstetter, Ding, Powers, & Salvi, 1997b; Salvi, Ding, Wang, & Jiang, 2000b).

Cochlear synaptopathy, a form of damage to the synapses between the inner hair cells and the ANFs is believed to be seen in individuals with noise exposure as well as due to ageing. This has been termed hidden hearing loss because, it is still unknown whether even humans showcase with similar deficits and also the effects are not exposed in any of the clinical tests, both in behavioural and physiological measures for absolute threshold (Oxenham, 2016). Animal studies have shown that noise exposure and ageing can cause loss of a large percentage of auditory nerve fibers (ANF) without significant change in their behavioural thresholds when tested with pure-tone audiometry (Bharadwaj et al., 2015). Lobarinas et al. (2013) conducted an experiment to assess the carboplatin induced IHC loss in chinchillas and to know the effects of it in pure-tone audiometry. They found a very little effect on audiometric thresholds even with very extensive inner hair cell loss which exceeded 80%, suggesting that conventional audiometry in not sensitive to inner hair cell loss and only a small number of these hair cells are required to detect a sound in quiet. Due to these limitations with the conventional audiometry, it is difficult to identify an individual with noise exposure or an aged individual who is at risk of further damage to the auditory structures which lead to the use of OAEs and ABR for assessing the initial damage seen in them.

2.1. Effects of Noise exposure on OAEs

In case of permanent damage to hair cells or damage to the mechano-sensory function, the more widely used diagnostic tests include pure-tone audiometry and OAEs which would reveal an increment in the thresholds and a decrement in the amplitude or absence of OAEs in those damaged frequency regions. However, OAEs

when compared to pure-tone audiometry has a better sensitivity in identifying the damage to the auditory structures (Attias, Horovitz, El-Hatib, & Nageris, 2001).

Vinck, Cauwenberge, Leroy, and Corthals (1999) studied the sensitivity of DPOAEs in monitoring the effects of TTS on OHCs. They exposed normal hearing individuals to a broad band noise of 90 dBSPL for one hour. DP-gram was recorded soon after the exposure and after 6 hours of exposure. The subjects were also made to undergo pure tone audiometry and the threshold was calculated from 250 Hz to 8000 Hz. It was found that the amplitude of DP-gram had significantly reduced soon after the exposure especially at frequencies 4000 Hz and above, even when audiometric thresholds were within the normal range. After six hours of termination of exposure, amplitude in DP-gram reverted back to normal. They concluded that changes due to exposure to noise are first seen in DPOAEs than in conventional audiometry. Hence, DPOAEs are more sensitive to noise induced changes compared to conventional audiometry.

It is identified that the Otoacoustic emissions are evoked by the OHCs within the cochlea, and this is the first site to be affected by noise exposure (Furst, Reshef, & Attias, 1992). In case of DPOAEs, wherein two pure-tone stimuli are presented, a notch at 3000 Hz is seen, resembling the configuration of hearing loss which is usually present as a notch at 4000-6000 Hz range (Attias et al., 2001). In the same study, there was a clear relationship between the OAEs and the thresholds that were obtained behaviourally. There was narrowing of the emission range and also a decrease in the amplitude of OAEs as the severity of the damage increased due to noise exposure. However, in few of the subjects with noise exposure, the OAEs were still present along with normal thresholds behaviourally (Attias et al., 2001).

Based on the work related to DPOAEs, it can be concluded that they serve as a reliable marker in monitoring the damage seen in cochlea in individuals with noise exposure. From the above mentioned studies, it can be noted that the DPOAEs assess the cochlear changes that are seen early with noise exposure and also to monitor the prolonged effect of the same. However, they do not account for early neural changes that might be associated with individuals exposed to occupational noise.

Later, morphological studies on animals revealed that there is a swelling of auditory nerve fiber (ANF) terminals at the site of connection with the hair cells i.e. the synaptic junction after acoustic overexposure (Liberman, 1982; Robertson, 1983; Spoendlin, 1971). And, this swelling was seen only at the synaptic connection area of the inner hair cells (IHCs), and not at the OHC area (Pujol & PUEL, 1999). This is also supported by many other studies revealing normal OHC functioning despite acoustic exposure. In one of the studies by Mehraei et al. (2015) done on human subjects, they assessed the changes seen due to acoustic exposure in different tests including inter-aural time difference (ITD), click-evoked otoacoustic emissions (CEOAEs), and ABR (analysis of wave I and wave V latency in the presence of masker noise). In normal hearing individuals without noise exposure the shift in wave V latency with increase in masker level was more compared to individuals with noise exposure and normal hearing. Also, the performance in sound localization task which required discrimination of ITDs in envelops of sound was better in without noise exposure group than with noise exposure group. Hence, it was seen that there could be damage at the synaptic level and not at the OHC region which was supported by the results obtained indicating a significant difference for the ITD and ABR measures and not for the CEOAEs (Mehraei et al., 2015).

Muller and Janssen (2008) conducted a study to find whether DPOAEs are a suitable measure for detecting small changes in the cochlear amplifier functionality due to occupational noise exposure. Measurements of contralateral suppression of DPOAEs using broad band noise (BBN) as the contralateral stimuli at 60 dB SPL was carried out. Results indicated a suppression of 1.6 dB on an average in individuals with occupational noise exposure whereas, a suppression of magnitude 1.9 dB in individuals without occupational noise exposure. Though there was a relatively lesser amount of suppression in individuals with noise exposure the difference was not significant. Hence, they established that DPOAEs are not a suitable means for detecting small changes in cochlear amplifier functionality due to occupational noise exposure.

Hence, we can conclude saying even with normal audiometric thresholds and presence of OAEs, there might be physiological changes seen at the neural level with or without cochlear damage which can be monitored through other tests assessing the brainstem or cortical level.

2.2. Effects of Noise exposure on ABR

Recent work on animals shows that overexposure to acoustic stimulation causing only transient threshold elevation, without any hair cell damage, nevertheless can cause irreversible loss of the synapses between inner hair cells and cochlear nerve fibers (Kujawa &Liberman, 2009). Furman et al. (2013) carried out an experiment on guinea pigs exposed to noise in 4000 Hz-8000 Hz octave bands at 106 dB SPL for 2 hours wherein, they recorded potentials from single auditory nerve fibers. They found that 2 weeks post-exposure, the ABR thresholds as well as the amplitude of DPOAEs recovered to normal, suggesting that there was recovery in the hair cell functioning. However, the supra-threshold ABR amplitudes had reduced and a loss of 30% of

synapses between the ANFs and inner hair cells were confirmed by Immunostaining pre and post synaptic markers of sensory epithelium. They concluded saying, this condition (cochlear synaptopathy) is selective for the subset of auditory fibers with high thresholds and low spontaneous rates (Furman et al., 2013). Evidence also shows that the difficulty with hearing in everyday setting and in understanding speech in noise with normal hearing could be due to the differences in the fidelity with which supra-threshold sound is coded in the auditory pathway (Bharadwaj et al., 2015). Cochlear synaptopathy due to noise-exposure has been studied extensively in animals wherein, there is a reduction in the amplitude of ABR wave I at the supra-threshold levels and not significant at the threshold level (Kujawa & Liberman, 2009; Lin et al., 2011; Hickox, Larsen, Heinz, Shinobu, & Whitton, 2017). However, there are very few studies to see whether the same results holds good for humans as well.

Prendergast et al. (2017) did a study on young human adults with a wide range of noise-exposure and normal hearing when tested through audiometry. ABR was done for high-pass filtered clicks (> 1500 Hz) at 80 and 100 dB peSPL. The bandwidth chosen was 3000 Hz -6000 Hz for the ABR stimuli and also the carrier frequency of transposed tones as this frequency region is commonly associated with damage due to noise-exposure in humans. They found that, there was no relation between the noise-exposure and the amplitude of ABR waves, especially wave I, which was seen in animals when exposed to noise. But, there was an increase in the latency of wave V of ABR when click stimuli was presented at 80 dB peSPL. They concluded saying these effects were not seen when the age was controlled, and therefore ABR is insensitive to this condition. However, these effects might become pronounced when there is an increase in age.

Almadori et al. (1998) studied any possibility of retro cochlear pathology in individuals with noise exposure having noise induced hearing loss. The study consisted of 54 (108 ears) individuals exposed to occupational noise at least for few years and having bilateral and symmetric sensorineural hearing loss at 1000 to 4000 Hz region. They recorded ABR for two stimulation rates 21/sec and 51/sec at 70 dB nHL for clicks of alternating polarity. They assessed the waveform morphology; absolute latencies for I, III and V peak and inter peak latencies for I-III, III-V and I-V. The results revealed absolute latencies and inter peak latencies were within normal limits. They observed poor waveform resolution especially in the peak I for 12 ears. The results also showed an absence of ABR in five ears which was not according to the loss of hearing. From results of this study we can conclude that hearing loss due to noise exposure might also have some neural correlate but has to be probed more in depth to know how the changes in auditory pathway are seen with time.

2.3. Effects of Ageing on OAEs

Previous research has shown that there is reduced amplitude of OAEs with ageing (Strouse, Ochs, & Hall, 1996). Most of the initial studies on age related hearing loss focused on the deterioration of hair cells and an increase or worsening of the thresholds (Gates & Mills, 2005). Changes in the inner ear and central auditory pathways are, however, the primary source of hearing impairment in the elderly. Histopathologic studies have documented degeneration of the sensory hair cells and supporting cells (Johnsson & Hawkins, 1976), degeneration of the striavascularis (Pauler, Schuknecht, & White, 1988), decrease in the number of functional spiral ganglia and 8^{th} nerve fibers (Wright & Schuknecht, 1972), loss of elasticity of the basilar membrane (Schuknecht et al., 1974), thickening of the tectorial membrane (Schuknecht et al., 1974), and a reduced cochlear blood supply (Jorgenson, 1961).

Lonsbury-Martin, Cutler, and Martin (1991) measured DPOAEs in 60 ears from individuals ranging in age from 31 to 60 years. Their findings revealed a tendency for older ears to generate smaller amplitude DPOAEs, particularly at the highest frequencies. Although their mean data showed audiometric thresholds equal to or better than 20 dB HL for all the groups, there was a large range in some groups. All subjects within the 30 to 40 year age range had audiometric thresholds less than or equal to 20 dB HL between 250 to 8000 Hz. In the older groups, however, 7 of 10 subjects had elevated thresholds at 3000, 4000, and 8000 Hz. Thus, as with the earlier studies which were even performed using TEOAEs, there was a significant age effect on audiometric thresholds.

A study done by Strouse et al. (1996) provided a differing outcome. They performed a study to re-evaluate the contribution of age and peripheral hearing loss on the prevalence and amplitude of DPOAEs by controlling for degree of peripheral hearing loss. Twenty subjects were divided into four age ranges. All subjects in each group had 15 dB HL or better thresholds from 250 Hz through 8000 Hz and normal immittance findings. DPOAE audiograms recorded at three intensity levels and input/output functions recorded at six discrete frequencies showed no significant differences in amplitude or noise level between age groups. Findings indicate that that when the degree of peripheral hearing loss is adequately controlled, there is no direct effect of advanced age on DPOAE measures. Although pooled findings reveal no overall significant differences in DPOAE measures between groups, there were several data points along the 65 dBSPL DPOAE audiogram and 1000 Hz I/O function for which group differences were present. But, these small number of data points showing significant group differences were not consistent across frequency or intensity levels and occurred at isolated frequency points. Hence, the results were

considered as artifactual at these frequencies. The authors explained the outcomes in support with previous studies wherein, if age has a direct effect on DPOAE amplitude, there would be a decreased DPOAE magnitude primarily for higher frequency regions among the elderly adults since these frequencies are the first to be affected in presbycusis. However, in the present study the group consisting older individuals showed significantly lower amplitude DPOAEs versus younger groups in regions at or below 1000 Hz as opposed to higher frequency regions. Sergeyenko et al. (2013) used Immunostaining technique to count the number of hair cells, synapses and spiral ganglion neurons (SGNs) in age graded series of mice. They got similar results to that of noise exposed mice wherein, the synapse between the IHC and the ANF; and the SGNs were the most vulnerable and damaged elements compared to the hair cells. The synaptic and the SGN loss increased monotonically from the initial age of testing i.e. four weeks till the time of death i.e. 144 weeks. However, the loss of hair cells was minimal when compared to the synaptic and SGN loss. During the intermediate stage, that is at the 80^{th} week of testing, the hair cell loss was approximately <5% when compared to almost 25% of synaptic loss at the same stage (Sergeyenko et al., 2013).

Therefore, in line with the above mentioned studies, it is clear that even in aged individuals, similar to noise exposed individuals, there is damage at the synaptic level which can be probed into by using tests that evaluate the brainstem structures like ABR. These studies also supports that cochlear damage (OHCs) which is evident in OAEs may not be the early structures to get damaged.

2.4. Effects of Ageing on ABR

Age-related hearing loss is a complex state and reflects pathologic changes along the entire auditory neuraxis. Difficulty in understanding speech, diminished

abilities to localize sounds, and a reduced ability to detect and extract target signals from noise are characteristic problems faced by the elderly. Central (neural) presbycusis frequently results in a striking loss in understanding speech without a significant or no change in the pure-tone thresholds when assessed audiometrically. In spite of evidence these deficits cannot be fully explained by peripheral changes alone, and hence, few works have examined the neurochemical basis of central auditory dysfunction in ageing. Age-related alterations in neural circuits involved in the processing of acoustic information could reflect changes in the synthesis, degradation, uptake, release, and receptor sensitivity of neurotransmitters, perhaps secondary to cell loss and/or progressive deafferentation (Caspary, Milbrandt, &Helfert, 1995).

A series of studies designed to test this hypothesis has examined ageing in central auditory system of the F344 rat. Age-related changes associated with GABA neurotransmitter function in an important auditory midbrain structure, the inferior colliculus, have been investigated. These studies found that there was a decrease in the number of GABA immuno-reactive neurons; decreased basal levels of GABA; decrease in GABA release; reduced glutamic acid decarboxylase activity; decreased GABA receptor binding; reduced number of presynaptic terminals; and subtle GABA receptor binding changes. Altogether, these age-related changes suggest altered GABA neurotransmitter function in the inferior colliculi (IC) (Caspary, Milbrandt,& Helfert, 1995).

A study was carried out by Boettcher, Mills, Norton, and Schmiedt (1993) to investigate the effects of ageing on auditory brainstem responses in young (6-10 month) and aged (36 month) Mongolian gerbils. ABR response amplitude and thresholds were measured at octave intervals from 1 till 16,000 Hz for each subject. The baselines were obtained from young animals to compare with the older animals

which were classified on the ABR thresholds. The four groups to which the older animals were classified included animals with thresholds (1) at the mean of a pool of 50 aged gerbils, (2) one standard deviation (SD) lower than the mean, (3) one SD higher than the mean, and (4) near normal for young gerbils. The outcome of ABR measures revealed a reduction in the amplitude of ABR waveforms for the aged gerbils when compared to the younger ones, particularly at high sound pressure levels. This result was true even for aged gerbils with thresholds similar to those of younger subjects. Further, the slopes of amplitude-intensity (I/O) functions were shallower in all aged subjects compared to younger subjects. The authors attributed the results to age related pathology in the auditory periphery and that the ABR amplitudes and I/O slopes decrease as a function of age and such reductions are not a direct result of loss of auditory sensitivity. The same authors with same set of subjects also reported that the wave I-IV interval was reduced or shorter in aged subjects with normal hearing than the normal group which served as the control; the interval was normal in aged subjects with 10-30 dB of loss; and increased or prolonged in subjects with greater than 30 dB of loss (Boettcher, Mills, Norton, & Schmiedt, 1993).

In clinical studies, high stimulus repetition rates have been used to improve the identification of the central auditory pathology. In a study conducted on Fischer 344 rat demonstrating both peripheral hearing loss and changes in auditory brainstem neurochemistry with age, interactions between stimulus level and repetition rate were examined. They examined the mono-aural threshold and standard ABR morphology in young (3-6 months) and old (20-23 months) rats using clicks at 10/s, with intensity varied from 0-100 dB. The effects of increasing stimulus repetition rate on ABR latency and morphology were evaluated at 0-100 dB using rates of 5, 10, 20, and 40/s. Old animals demonstrated elevated ABR click thresholds, reflected by shifts in the

latency-intensity curves. With increased stimulation rates, aged rats exhibited prolonged wave IV and V latencies, especially at the highest intensities, with degraded waveform morphology. Peak amplitudes were generally reduced for older group, irrespective of rate or the stimulus level. They suggested that the auditory processing will be altered in aged animals, while the selective effects of rate increases on waves IV and V provide supporting evidence for possible involvement of the central auditory generators of these components (Backoff & Caspary, 1994).

Jerger and Hall (1980) examined the latency and amplitude of the wave V ABR waveform as a function of chronological age in 182 male and 137 female subjects. Out of 319 subjects, in 98 of them the hearing thresholds were within normal limits and the remaining 221 subjects had varying degrees of sensorineural hearing loss. It was seen that age had a very less effect on both latency and amplitude of wave V. In case of normal hearing subjects, wave V latency increased with age from 25 to 55 years by about 0.2 ms and amplitude decreased by about 10%. In subjects with sensorineural hearing loss the effects seen were less, i.e. the latency increase was smaller, whereas the amplitude decrease was equivalent.

Evidence also shows that in animals the amplitudes of early waves, but not late waves, decrease greatly with ageing (Hunter & Willott, 1987). A study was conducted in young and old (C57BL/6J) mice, wherein the amplitude and latencies of all the ABR waves across intensity were assessed with filtered noise pips. Presbycusis, in ageing C57 mice, is associated with increased thresholds; there is a trend toward increased latencies, but only when threshold elevations are substantial. Results obtained indicated that amplitudes of early waves, but not late waves, decrease greatly in ageing C57 mice. In young C57 mice, amplitudes of early ABR waves vary monotonically with intensity, while amplitudes of later waves have a

relatively flat or even non-monotic, relationship to intensity; in older C57 mice, all waves have monotonic intensity functions (Hunter & Willott, 1987).

In 1999, Walton, Orlando and Burkard investigated ageing effects on ABR wave V latency using a tone-on-tone burst forward masking paradigm. They found that at short forward masking intervals, wave V latency shift was greater in normal-hearing older adults than in normal-hearing young adults for moderate level, high frequency toneburst maskers and probes. It was not possible to evaluate wave I latency because stimulation and recording procedures did not produce a consistently observable wave I. In order to optimize the recording of wave I, they used a high-level (115 dB pSPL) click stimulus, combined with a tympanic membrane inverting electrode, and investigated the latencies and amplitudes of wave I and V across click rate. Young adults had hearing thresholds within normal limits, whereas older adults had normal or mild threshold elevation. ABRs were obtained at click rates of 11, 25, 50, and 75/sec. Using maximum length sequences (MLSs), ABRs were recorded at 100, 200, 300, 400, and 500 Hz. Results across age groups were very similar i.e. with increasing click rate, peak latencies increased, the I-V interval increased and peak amplitudes decreased. The most notable difference between age groups was that wave I amplitude was substantially smaller in the older subjects. They concluded that changes in the ABR with increasing rate are remarkably similar in young and older adults when audiometric thresholds are normal or near-normal in both age groups.

2.5. Combined effects of ageing and noise exposure on the auditory system

Ageing which causes a decreased efficiency in the functioning of a system is usually associated with a down-regulation in metabolism. This can lead to an increased sensitivity to stress agents and also a decreased repair of tissues. When this is considered in the auditory system, it can result in an increased sensitivity to noise

induced hearing loss (NIHL) with age. Miller, Dolan, Raphael, and Altschuler (1998), conducted a study to assess the combined or the interactive effects of ageing and noise exposure in mice. The mice were exposed to high intensity noise of 108 dB SPL for 45 min at 500 -40000 Hz. They included normal young and old mice (CBA/Ca) along with young premature presbycusis (C57BL/6) mice. Tone evoked ABR thresholds were obtained before and after the exposure along with cyto-cochleograms. Results indicated an increase in the threshold shifts and hair cell losses after the noise exposure with increasing age of the mice. Also, in mice which showed early presbycusis associated with vascular pathology, there was an increased sensitivity to NIHL compared to the normals. They also found that in some of the young premature presbycusis (C57BL/6) mice, the physiological loss was not associated with hair cell loss. These findings support the view that ageing with or without hearing loss increased the sensitivity of the ear to NIHL. Kujawa and Liberman (2006) did a study to see the interaction between ageing and early noise exposure in mice. They found that exposure to noise which was designed to cause a permanent threshold shift (PTS) in both DPOAEs and ABR, suffered a delayed loss of spiral ganglion neurons (SGNs) ranging from months to years of post-exposure without any initial or delayed loss of either OHCs or IHCs. It was concluded that, sub clinical but still pathological changes due to early noise exposure can cause more vulnerability of the hearing system during the process of ageing.

Thus, further research on how the auditory system changes in aged individuals and in individuals with noise exposure is required in humans to delineate the patho-physiology.

Chapter 3

Method

The study was carried out to compare DPOAEs and ABR in individuals with noise exposure and aged individuals, with normal hearing at different presentation levels and to know which among the ABR parameters including latency of wave I and wave V; amplitude of wave I and V; and wave V/I ratio was a better indicator of ANF loss.

3.1. Selection of participants

Thirty adult male participants divided into three groups of ten individuals each, were considered for the study. Group 1 includedindividuals not exposed to occupational noise with <35 yearsof age(N=10) andalso served as the control group.Individuals aged >45 years without any occupational noise exposure formed Group 2(N=10).Group 3 included individuals who are exposed to noise greater than 80 dB(A) for a duration of 8 hours per day in their work place with age <35 years (N=10). The mean age and age range of the individuals considered for the study is provided in Table 3.1. The individuals considered for the study had a flat audiometric configuration that is less than 5dB rise or fall per octave as given by Silman & Silverman (1991). The subjects gave a written consent prior to the evaluations.

As a criterion for selection, the hearing threshold of the subjects in each of the groups were within normal range of <25dB HL at all four octave frequencies (500Hz, 1000 Hz, 2000Hz& 4000Hz).

3.1.1. Exclusion criteria. Participants who presented with any of the conditions were excluded from the study:

- any history or presence of middle ear disorders
- any psychological or neurological dysfunction
- presence of tinnitus
- if they were smokers or alcoholics
- if they were under any medications for other ailments
- if they were using any type of ear protective devices
- if they were exposed to loud music/ use earphones for a longer duration on a daily basis

3.2. Test environment

All the participants were subjected to tests in an acoustically treated room where the ambient noise level was within the permissible limits as specified by ANSI S3.11999 (R 2008).

3.3. Procedure

3.3.1. Preliminary evaluations. As a first step, a detailed case history was taken from all the participants to rule out any pathological conditions of auditory system and to procure information about their working environment and work experience. All participants were subjected to pure tone audiometry using Inventis Piano a dual channel audiometer coupled to TDH 39 earphones with MX-41/AR ear cushions for octave frequencies between 250 to 8000 Hz to estimate the air conduction threshold and a bone vibrator (Radio ear B-71) for testing the bone conduction thresholds. The threshold was estimated using modified Hughson and Westlake procedure (Carhart & Jerger, 1959) in a sound treated room. The 25 dB HL threshold criteria was fixed in order to rule out any peripheral hearing loss in the participants. The mean pure-tone averages for all the three groups are provided in

Table 3.1. Speech recognition thresholds were obtained using Kannada paired words and Speech Identification Scores (SIS) using Phonetically Balanced (PB) word lists in Kannada language (Yathiraj & Vijayalakshmi, 2005).Immittance evaluation which includes both tympanometry and acoustic reflexes was done to rule out any middle ear dysfunction. Acoustic reflex using 226 Hz probe tone at 500 Hz, 1000 Hz, 2000 Hz,& 4000Hz was assessed using GSI-Tympstar middle ear analyzer. Individuals who had normal acoustic reflexes at the above mentioned frequencies were considered for the study.

Participants satisfying the above mentioned selection criteria were included for further evaluations.

Table 3.1

Mean pure tone average, mean age and age range of subjects participated in the study

		Number of subjects	Age (in years)		Mean pure-tone average (in dB)
			Mean	Range	
Control group		N=10	30.0	28-34	7.075
Clinical group	Aged individuals	N=10	53.2	45-65	7.825
	Noise-exposed individuals	N=10	32.4	29-35	7.197

3.3.2. DPOAEs measurements. DPOAE fine structure was studied at 8 points per octave to assess the functioning of the outer hair cells. The stimulus parameters used to record DPOAEs are as follows:

- f2 frequencies: 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, and 8000 Hz.
- f2/f1 ratio: the ratio used to elicit DPOAEs was 1.22 as it provides optimal DPOAE amplitude (Harris, Coats,& Martin, 1999).

- L1 and L2 levels: the f1 and f2 primaries were presented at 65 dB SPL and 55 dB SPL respectively as it provides lesser artifacts and optimum results (Stover, Gorga, Neely, & Montoya, 1996).

The DOPAEs were evaluated for amplitude parameter at various DP frequencies and the signal to noise ratio (SNR) was recorded. The responses were considered to be present if the SNR exceeded 6 dB (Gorga et al., 1993).

3.3.3. ABR recording. The ABR was done in a sound treated room using Biologic Navigator Pro system (version 7.2.0.). The potentials were obtained with electrodes placed at Fz, M1, M2; and ground at Fpz position (vertical montage). The electrode impedance considered was below 5kΩ at all the electrodes. The stimulus was presented through ER-3A insert earphones. The stimuli used for assessment was click and the level was decreased in 10dB steps from 90dBnHL to 50 dBnHL. The level was not reduced further because, wave I is absent in most of the individuals as it nears the threshold (Stamper & Johnson, 2015). A repetition rate of 7.1/sec was considered as it provides good representation/morphology of the waveform at lower levels of stimulus presentation (Paludetti, Maurizi, & Ottaviani, 1983; Backoff & Caspary, 1994). A band pass filter of 100-3000 Hz was used and collected in a 12 ms time window. Two thousands sweeps were averaged at each presentation for two replications and the average was taken. The absolute amplitude and absolute peak latencies for wave I and V; and wave V/I ratio were analyzed for all the groups at only high levels of presentation (90 dB, 80 dB and 70 dBnHL) since wave I is not prominent for all at lower levels of presentation. However, at lower levels (60 dB and 50 dBnHL) only wave V latency was analysed.

The analyses of the waveforms were performed for all the participants wherein, the peak identification and morphology rating were done by two experienced

audiologists in wave form analysis. Both the audiologists were blinded to subject information during the waveform analysis process. The peaks that were marked by one audiologist and not by the other audiologist was not considered for the study. The ABR measures considered for the analysis were absolute latency, absolute amplitude, and peak V/I amplitude ratio. The peaks considered were marked as I, III, and V. The latencies of the peaks were calculated by taking the center or midpoint when the waveforms contained double peaks of equal amplitude and was marked at the centre of the larger peak when the peak were unequal in amplitude. The amplitude of each peak was defined as the largest positive or negative deflection depending on whether it's a negative or positive peak in the response window (Konrad-Martin et al., 2012).

Table 3.2

Stimulus and acquisition parameters for recording ABR

Stimulus parameters	
Transducer type	ER-3A Insert headphone
Type of stimulus	Click
Intensity	Swept from 90dB nHL to 50 dB nHL
Stimulus polarity	Rarefaction
Stimulus rate	7.1/s
Acquisition parameters	
Analysis time	12ms
Gain	100000
Filter setting	100-3000Hz
No of sweep	2000
Electrode montage	Inverting(-) = Test ear mastoid Non inverting(+)= Non-test ear mastoid Ground = Forehead (Fz)

3.4. Statistical Analyses

The data was analysed using Statistical Package for the Social Sciences (SPSS). Shapiro-Wilks test of nomality was performed to determine whether the data was normally distributed or not. The ABR amplitude and latency parameters; and DPOAEs amplitude between the groups were statistically analyzed.

Chapter 4

Results

The aim of the present study was to investigate the effects of ageing and noise exposure on the auditory system using different tests including DPOAEs and ABR. The measures used for analysis in ABR included latency of wave I and V; amplitude of wave I and V; and wave V/I amplitude ratio. Also, the amplitude of DPOAEs at different frequencies were analyzed to check for any differences. The ABR waves were recorded at five different intensities including 90, 80, 70, 60 and 50 dB nHL. The responses from these intensities were compared between the three groups namely, individuals without occupational noise exposure with age less than 35 years who served also as the control group (Group 1); aged individuals in the age range of 45-65 years without occupational noise exposure (Group 2); and, individuals with noise exposure with age not more than 35 years (Group 3), all having normal hearing thresholds when assessed audiometrically.

Shapiro-Wilks test of normality was administered to check whether the data follows normal distribution for both ABR and DPOAE measures. It was found that ABR parameters studied did not follow normal distribution ($p< 0.05$) and hence non-parametric tests were administered whereas, DPOAEs data did follow normal distribution ($p> 0.05$)and therefore, parametric tests were administered. The variability is accounted to the heterogeneity in the participants of the study. The statistical tests administered are as follows:

1. Descriptive statistics was performed to examine the central tendency and variation of latency and amplitude parameters of ABR and amplitude of DPOAEs among the participants studied.

2. Kruskal-Wallis test was administered to compare the latency and amplitude parameters of ABR between the three groups.
3. Mann-Whitney U test was performed for parameters which exhibited a significant difference in Kruskal-Wallis test.
4. MANOVA test was done to compare the amplitudes at different frequencies in DPOAEs between the three groups.
5. As a part of post-hoc analysis, Duncan's test was carried out for those parameters that showed a significant difference in DPOAE amplitudes across frequencies.

4.1. Comparison of latency of ABR waves between the groups

Descriptive statistics were carried out to find the mean, median and standard deviation of wave V, III and I of Group 1 (Control group), Group 2 (Aged individuals without occupational noise exposure) and Group 3 (individuals with occupational noise exposure) at different presentation levels ranging from 90 dB nHL to 50 dB nHL, reduced in 10 dB steps for latency parameter and the same is provided in Table 4.1.

Table 4.1

Mean, Median and SD of Group 1, Group 2 and Group 3 for latency parameter for wavesI, III and V at different levels (90-50 dB nHL)

Stimulation Level	Waves I, III & V;(N)	Latency parameter								
		Group 1			Group 2			Group 3		
		Mean (ms)	Median (ms)	SD	Mean (ms)	Median (ms)	SD	Mean (ms)	Median (ms)	SD
90 dB nHL	I (N= 30)	1.30	1.28	0.07	1.64	1.62	0.13	1.65	1.59	0.15
	III (N= 30)	3.53	3.53	0.13	3.58	3.61	0.07	3.54	3.55	0.08
	V (N= 30)	5.36	5.3	0.15	5.46	5.49	0.11	5.31	5.24	0.20
80 dB nHL	I (N= 28)	1.49	1.43	0.22	1.61	1.62	0.12	1.59	1.62	0.12
	III (N= 30)	3.56	3.55	0.12	3.65	3.66	0.09	3.60	3.61	0.07

	V (N= 30)	5.44	5.4	0.12	5.61	5.63	0.18	5.44	5.42	0.21
70 dB nHL	I (N= 25)	1.60	1.57	0.23	1.76	1.65	0.11	1.67	1.72	0.07
	III (N= 29)	3.63	3.59	0.16	3.57	3.6	0.11	3.70	3.72	0.09
	V (N= 30)	5.58	5.61	0.14	5.64	5.65	0.14	5.58	5.61	0.23
60 dB nHL	III (N= 25)	3.82	3.74	0.19	4.01	3.97	0.28	3.92	3.93	0.16
	V (N= 30)	5.77	5.72	0.24	6.16	6.09	0.25	5.76	5.77	0.26
50 dB nHL	III (N= 18)	4.00	3.95	0.16	4.34	4.32	0.11	4.15	4.07	0.17
	V (N= 30)	5.96	5.97	0.25	6.55	6.47	0.28	6.15	6.19	0.32

Note. The descriptive statistics at 50 and 60 dB nHL for wave I were excluded from the statistics and not included in the table as very few number of subjects exhibited this response (N < 3 from each group).

The descriptive statistics results of latency parameter indicate that there was an increase in the mean latency of different ABR waves including I, III and V at all the tested intensity levels for both Group 2 and Group 3 when compared with Group 1. The same is depicted in Table 4.1. It was observed that the prolongation of waves I, III and V were slightly more in Group 2 compared to Group 3 when mean latencies were analyzed using descriptive statistics. The averaged waveform across the three groups at different intensities is represented in Fig 4.1. to Fig 4.5. Kruskal-Wallis Test was administered to compare the three independent groups for latency parameter at different intensities. The Kruskal-Wallis test indicated a significant effect, $\chi^2(2) = 18.79$, $p < 0.05$ at 90 dB nHL for wave I; $\chi^2(2) = 10.06$, $p < 0.05$ at 60 dB nHL for wave V; $\chi^2(2) = 13.75$, $p < 0.05$ and $\chi^2(2) = 7.52$, $p < 0.05$ at 50 dB nHL for wave V and wave III respectively.

Further, Mann-Whitney U test was administered to check for the difference between two independent groups wherein parameters that showed a significant difference in Kruskal-Wallis test were analyzed. It was observed that there was a significant difference in latency, $|Z| = 3.78$, $p < 0.05$ at 90 dB nHL for wave I; $|Z| =$

2.73, $p < 0.05$ at 60 dB nHL for wave V; $|z| = 3.52$, $p < 0.05$ and $|z| = 2.39$, $p < 0.05$ at 50 dB nHL for wave V and wave III respectively between Group 1 and Group 2 whereas, the difference, $|z| = 3.57$, $p < 0.05$ was present only for latency of wave I at 90 dB nHL, between Group 1 and Group 3. When Group 2 and Group 3 were compared the difference was evident only for wave V, $|z| = 2.01$, $p < 0.05$ at 50 dB nHL; and $|z| = 2.67$, $p < 0.05$ at 60 dB nHL for latency parameter. The wave I latency at 50 and 60 dB nHL was excluded from the statistical analyses as the number of subjects who demonstrated a response at that intensity were very few (N < 3 in each group). Hence, it is difficult to compare wave I latency measure between groups at such lower intensity levels even though it is considered as a supra-threshold level.

4.2. Comparison of absolute amplitude of ABR waves and wave V/I ratio between the Groups

Descriptive statistics were carried out to find the mean, median and standard deviation of peak V, III and I of Group 1, Group 2 and Group 3 at different presentation levels ranging from 90 dB nHL to 50 dB nHL in 10 dB steps for amplitude parameter and the same is provided in Table 4.2. It was also found in the descriptive statistics that the mean V/I amplitude ratio was higher for Group 2 and Group 3 compared to Group 1. The result of the same is provided in Table 4.2.

Table 4.2

Mean, Median and SD of Group 1, Group 2 and Group 3 for amplitude parameter for wavesI, III and V; wave V/I amplitude ratio at different levels (90-50 dB nHL)

Stimulation Level	Waves I, III & V; V/I ratio; (N)	Amplitude parameter								
		Group 1			Group 2			Group 3		
		Mean (µV)	Median (µV)	SD	Mean (µV)	Median (µV)	SD	Mean (µV)	Median (µV)	SD
90 dB nHL	I N = 30	0.38	0.39	0.13	0.18	0.17	0.03	0.17	0.17	0.05
	III N = 30	0.42	0.34	0.17	0.22	0.22	0.06	0.43	0.40	0.17

	V N = 30	0.56	0.51	0.22	0.46	0.48	0.06	0.59	0.53	0.24
	V/I N = 30	1.58	1.58	0.65	2.64	2.65	0.45	2.77	2.93	0.44
80 dB nHL	I N = 28	0.34	0.34	0.10	0.16	0.16	0.03	0.16	0.17	0.03
	III N = 30	0.36	0.35	0.08	0.25	0.22	0.08	0.34	0.35	0.03
	V N = 30	0.43	0.36	0.23	0.35	0.34	0.09	0.37	0.40	0.10
	V/I N = 28	1.25	1.08	0.46	2.06	2.10	0.54	2.36	2.52	0.87
70 dB nHL	I N = 25	0.35	0.34	0.10	0.11	0.10	0.03	0.12	0.13	0.02
	III N = 29	0.25	0.21	0.08	0.14	0.15	0.05	0.25	0.27	0.06
	V N = 30	0.37	0.34	0.12	0.29	0.31	0.06	0.30	0.26	0.11
	V/I N = 25	1.09	1.02	0.26	2.68	2.10	0.64	2.24	2.54	0.76
60 dB nHL	III N = 25	0.18	0.17	0.08	0.14	0.15	0.04	0.18	0.17	0.07
	V N = 30	0.28	0.29	0.07	0.22	0.20	0.06	0.18	0.18	0.02
50 dB nHL	III N = 18	0.13	0.11	0.08	0.13	0.12	0.05	0.08	0.08	0.02
	V N = 30	0.26	0.27	0.08	0.15	0.14	0.04	0.17	0.15	0.05

The descriptive statistics results of amplitude parameter indicate that there was a decrease in the mean amplitude of different ABR waves including I, III and V; and an increase in wave V/I amplitude ratio at most of the tested intensity levels for both Group 2 and Group 3 when compared with Group 1. It was observed that the reduction in amplitude of waves I, III and V was more in Group 2 compared to Group 3, when mean amplitudes of different waves were analyzed using descriptive statistics. It was also noted that the mean amplitude of wave V for Group 3 was similar to Group 1 especially at higher stimulation levels of 90, 80 and 70 dB nHL. In other words, a more pronounced difference was seen for wave I amplitude when compared to wave III and wave V amplitudes. The averaged waveform across the three groups at different intensities is represented in Fig 4.1. to Fig 4.5.

Kruskal-Wallis Test was administered to compare the three independent groups for amplitude parameters at different intensities. This test indicated a

significant effect for wave I amplitude and wave V/I amplitude ratio at higher intensity levels. The number of subjects who exhibited wave I response and wave V/I amplitude ratio were very less (N < 3 in each group) at 50 and 60 dB nHL and hence, were excluded from the statistical analyses. However, the difference was significant at only very few selected intensities for wave III and V which did not follow any trend. The Kruskal-Wallis test results for parameters that exhibited a significant difference is depicted in Table 4.3.

Further, Mann-Whitney U test was administered to check for the difference between two independent groups for the parameters that showed a significant difference in Kruskal-Wallis test. It was observed that there was a difference seen between Group 1 and 2; and, Group 1 and 3 in most of the parameters that exhibited a significant difference in Kruskal-Wallis test. However, there was no significant difference observed between Group 2 and Group 3. Mann-Whitney test results are depicted in Table 4.3 for parameters that showed a significant difference.

Table 4.3

Table depicting test values having significant differences in Kruskal-Wallis test and Mann-Whitney U test at different intensities between the three groups for amplitude parameters

Intensity	Parameters	Kruskal-Wallis H test	Mann-Whitney U test		
			Group 1 &Group 2	Group 1 &Group 3	Group 2 &Group 3
90 dBnHL	Wave III	$\chi^2(2) = 11.66$ $p<0.05$	|Z|= 2.99 $p< 0.05$		|Z|= -2.81 $p< 0.05$
	Wave I	$\chi^2(2) = 17.51$ $p<0.05$	|Z|= -3.72 $p< 0.05$	|Z|= -3.38 $p< 0.05$	
	Wave V/I	$\chi^2(2) = 13.63$ $p<0.05$	|Z|= -3.1 $p< 0.05$	|Z|= -3.11 $p< 0.05$	
80 dBnHL	Wave III	$\chi^2(2) = 8.04$ $p<0.05$	|Z|= -2.27 $p< 0.05$		|Z|= -2.54 $p< 0.05$
	Wave I	$\chi^2(2) = 17.84$ $p<0.05$	|Z|= -3.68 $p< 0.05$	|Z|= -3.42 $p< 0.05$	
	Wave V/I	$\chi^2(2) = 11.63$ $p< 0.05$	|Z|= -2.94 $p< 0.05$	|Z|= -2.73 $p< 0.05$	

70 dBnHL	Wave III	$\chi^2(2) = 10.11$ $p<0.058$	|Z|= -2.7 $p< 0.05$		|Z|= -2.74 $p< 0.05$
	Wave I	$\chi^2(2) = 16.39$ $p<0.05$	|Z|= -3.56 $p< 0.05$	|Z|= -3.07 $p< 0.05$	
	Wave V/I	$\chi^2(2) = 15.38$ $p<0.05$	|Z|= -3.56 $p< 0.05$	|Z|= -2.70 $p< 0.05$	
60 dBnHL	Wave V	$\chi^2(2) = 9.01$ $p<0.05$	|Z|= -2.12 $p< 0.05$	|Z|= -2.69 $p< 0.05$	
	Wave I	$\chi^2(2) = 8.22$ $p<0.05$		|Z|= -2.40 $p< 0.05$	
50 dBnHL	Wave V	$\chi^2(2) = 9.42$ $p<0.05$	|Z|= -2.8 $p< 0.05$	|Z|= -2.32 $p< 0.05$	

Note. The shaded space indicates no significant difference between the groups compared.

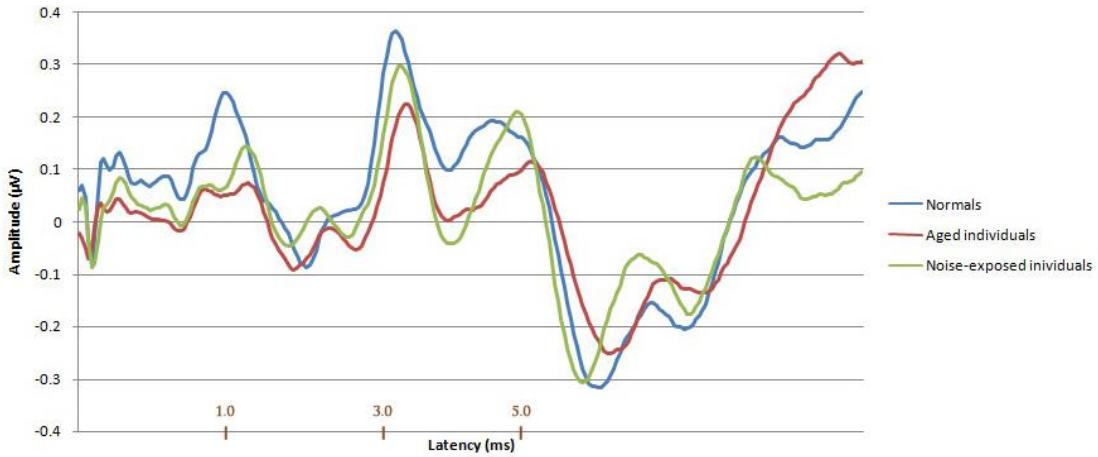

Figure 4.1 Averaged waveforms of three groups at 90 dB nHL.

Note. For latency parameter, a significant difference was observed for wave I between Group 1 and Group 2, and also between Group 1 and Group 3. For amplitude parameter, a significant difference was present for Group 2 and Group 3 when compared with Group 1 for wave I; between Group 1 and Group 2 and between Group 2 and Group 3 for wave III.

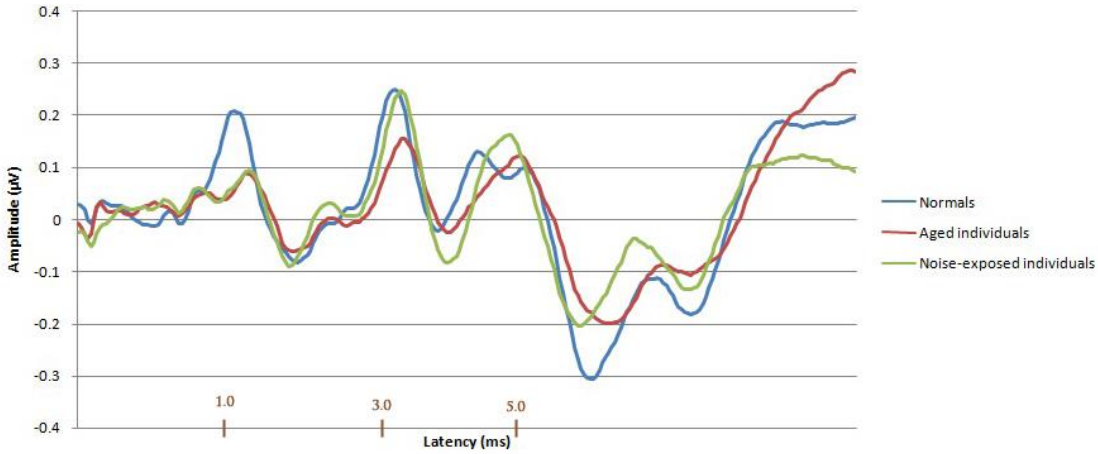

Figure 4.2 Averaged waveforms of three groups at 80 dB nHL.

Note. For amplitude parameter, a significant difference was present for Group 2 and Group 3 when compared with Group 1 for wave I; between Group 1 and Group 2 and between Group 2 and Group 3 for wave III.

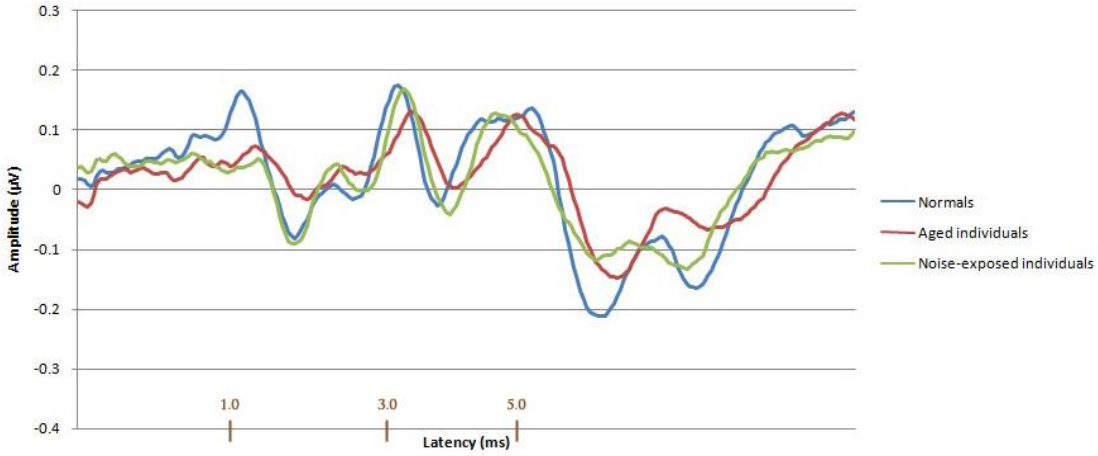

Figure 4.3 Averaged waveforms of three groups at 70 dB nHL.

Note. For amplitude parameter, a significant difference was present for Group 2 and Group 3 when compared with Group 1 for wave I; between Group 1 and Group 2 and between Group 2 and Group 3 for wave III.

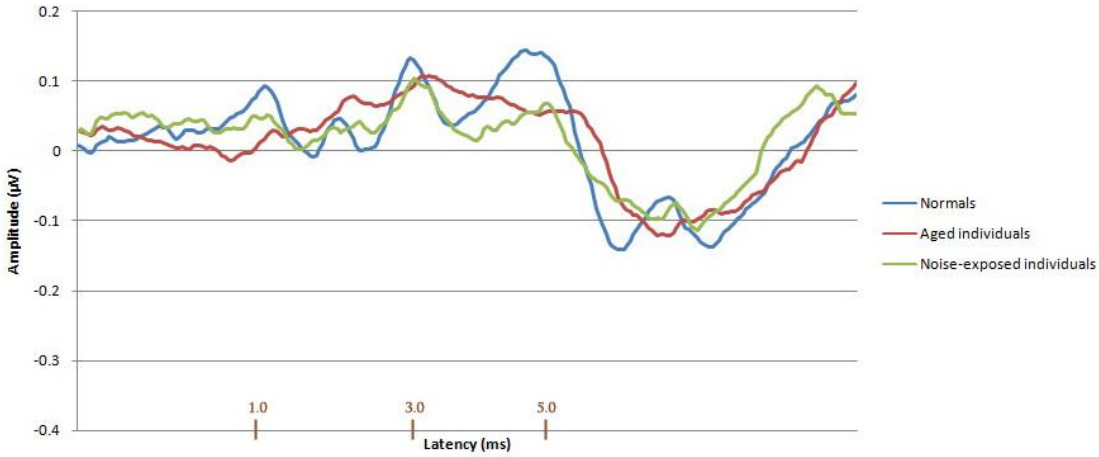

Figure 4.4 Averaged waveforms of three groups at 60 dB nHL.

Note. For latency parameter, a significant difference was observed for wave V between Group 1 and Group 2; and between Group 2 and Group 3. For amplitude parameter, a significant difference was present between Group 1 and Group 3 for wave I; between Group 2 and Group 3 when compared with Group 1 for wave V.

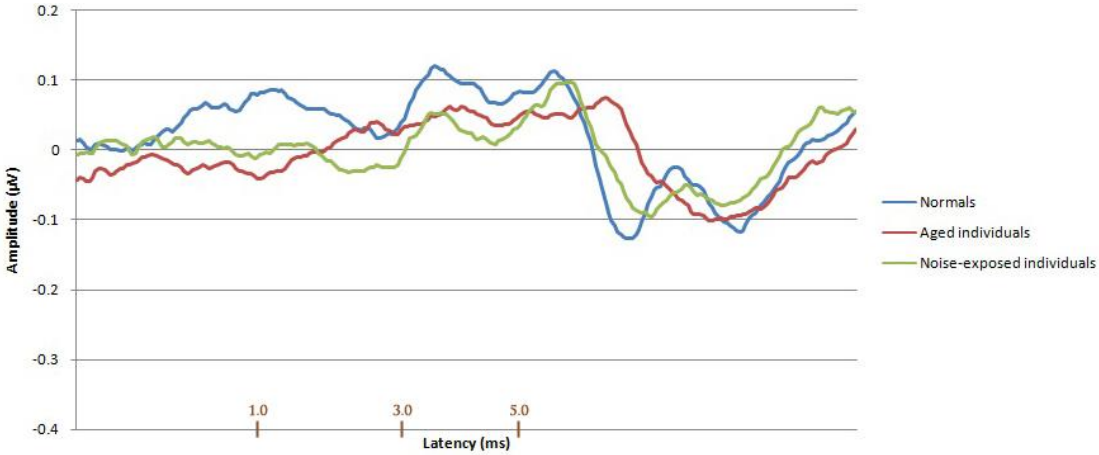

Figure 4.5 Averaged waveforms of three groups at 50 dB nHL.

Note. For latency parameter, a significant difference was observed for wave III and V latency between Group 1 and Group 2; and for wave V between Group 2 and Group 3. For amplitude parameter, a significant difference was present for Group 2 and Group 3 when compared with Group 1 for wave V.

4.3. Comparison of amplitude of DPOAEs between the Groups

Descriptive statistics for DPOAEs assessed at different frequencies indicated similar amplitude at almost all the frequencies tested in all the groups. The descriptive statistics for DPOAEs at different frequencies are provided in Table 4.4.

Table 4.4

Mean and SD for DPOAE amplitude across frequency in Group 1, Group 2 and Group 3

f2 Frequency (Hz)	DPOAEs amplitude					
	Group 1		Group 2		Group 3	
	Mean (dB)	SD	Mean (dB)	SD	Mean (dB)	SD
842	4.91	0.72	4.89	0.82	5.07	0.85
916	5.68	0.55	4.11	0.58	4.26	0.78
1001	4.51	0.61	5.05	0.73	4.96	0.81
1086	7.05	1.10	7.89	0.81	5.36	0.73
1184	8.60	1.90	5.64	1.34	4.21	0.55
1294	8.47	1.35	5.55	0.69	6.72	1.31
1416	10.23	2.82	7.92	1.19	9.11	1.29
1538	10.05	2.01	5.16	0.63	6.75	0.63
1685	11.23	3.21	7.00	1.32	8.38	1.38
1831	10.00	2.04	6.80	1.15	7.78	1.37
2002	8.43	1.62	7.45	1.86	6.11	1.60
2185	9.05	1.13	7.63	1.67	7.42	1.76
2380	10.01	2.57	5.82	1.79	7.47	2.10
2600	7.98	1.42	6.14	1.34	6.70	1.29
2832	8.14	0.76	5.57	0.98	6.32	1.97
3088	4.28	0.76	4.34	0.71	5.25	1.20
3369	6.01	1.97	4.77	0.72	4.58	0.63
3662	8.13	1.05	5.69	1.80	5.98	0.61
4004	9.02	2.78	8.32	1.45	7.76	1.80
4358	11.50	3.99	5.64	0.68	7.05	2.81
4761	4.61	0.73	4.12	0.78	4.68	0.55
5188	4.00	0.77	4.03	0.79	5.17	1.09
5652	5.22	0.54	4.05	0.65	4.42	0.92
6165	9.07	1.83	2.54	0.57	4.45	0.78
6726	7.14	1.15	-1.25	0.75	5.81	0.87
7336	5.55	1.11	1.86	0.62	4.17	1.10
7996	5.92	0.95	2.43	0.64	4.58	0.95

The data was subjected to normality test wherein, Shapiro-Wilks test of normality was used and found that most of the data followed normal distribution and hence, parametric test was used to check the level of significance among the groups. Multivariate Analysis of Variance (MANOVA) was the test used for the same. Before administering the parametric test all the outliers were removed from the raw data. MANOVA test revealed a significant difference among the three groups compared at only three frequencies that is, 4358 Hz, 6165 Hz and 6726 Hz. It was observed that

the amplitude of DPOAEs for Group 2 and Group 3 were reduced in comparison to Group 1 however, there was no significant difference observed between the groups. The MANOVA test results for frequencies that exhibited a significant difference is depicted in Table 4.5. Further, the results of MANOVA for frequencies where there was no significant difference is mentioned in Appendix 1.

Table 4.5

The MANOVA test results for frequencies that exhibited a significant difference in Group 1, Group 2 and Group 3

Frequency (Hz)	**F value**
4358	F (2, 48) = 9.97, p<0.05
6165	F (2, 48) = 6.13, p < 0.05
6726	F (2, 48) = 4.99, p < 0.05

A post-hoc analysis was carried out using Duncan's test for DPOAEs amplitude for the frequencies that showed a significant difference in MANOVA. Among all the frequencies that showed a significant difference in MANOVA that is, at 4358 Hz, 6165 Hz and 6726 Hz, there was no significant difference found between Group 2 and Group 3 (p > 0.05) whereas, a significant difference was present for Group 1 when compared with Group 2 and 3 (p < 0.05). In summary, the DPOAEs amplitude showed a significant difference at only 4358 Hz, 6165 Hz and 6726 Hz and no significant difference among the other frequencies tested.

Hence, the first hypothesis is accepted as there was no significant difference observed on both ABR and DPOAEs at various intensities tested between Group 2 and Group 3. The second objective is partially rejected as there was a significant difference observed when Group 2 and Group 3 were compared with Group 1 for the parameters of ABR and no difference across frequencies for DPOAEs.

Chapter 5

Discussion

The aim of the present study was to compare the supra-threshold measures like DPOAEs and ABR in two groups; individuals with noise exposure and aged individuals, with the normal hearing group. The results indicated that there was a significant difference observed in the amplitude parameter for wave I and wave V/I ratio compared to the amplitude of other waves and no significant difference in terms of latency parameter was noted. Also, the difference was not evident for amplitude of DPOAEs between the groups compared.

The responses of ABR showed a clear increase in latency of wave I, III and V with decrease in intensity level, consistent with previous report (Dau, 2003). Although, the latencies of Group 2 and 3 were slightly higher compared to Group 1, there was no significant difference observed between the groups except for very few waves (wave I at 90dB nHL; wave V at 60 dBnHL; waves III and V at 50 dBnHL). Previous work has shown that approximately 40% of the auditory nerve cochlear synapses could be destroyed permanently without any permanent threshold elevation for the auditory brainstem response, which is reflected by the summed activity of the auditory nerve fibers in its first wave (Melcher & Kiang, 1996). It is clear from the present study that there was no significant difference observed for latency parameter in all the intensities tested. This could be because, there is a reduction in the number of fibers firing which is evident as reduced wave I amplitude and not with the speed of transmission of the signal which is characterized by the latency.

The wave I amplitude at higher intensities to click stimuli were significantly smaller in ears with noise exposure and ageing when compared to the normals of age

< 35 years without occupational noise exposure, here on referred to as normals. At higher testing levels (>70 dB nHL), there was a systematic trend for wave I amplitude to decrease in aged and noise exposed individuals. This trend of reduced amplitude was not well established at lower intensity levels. This could be because, even in individuals with normal hearing the presence of wave I and III reduces at lower intensity levels and hence, it is difficult to use as an indicator. In contrast to the results obtained for wave I, there was no decrement in the wave V amplitude at supra-threshold levels.

Similar results were obtained by Stamper and Johnson (2015) wherein, the suprathreshold wave I amplitude was smaller in normal hearing group with greater noise exposure backgrounds when compared to normal hearing subjects with lesser nose exposure backgrounds. Comparable findings were obtained from animal studies also, yielding a reduced wave I amplitude in relation to the other waves (Kujawa &Liberman, 2009; Furman et al., 2013). It was stated by Furman et al. (2013) that the neural degeneration is seen initially as a loss of synapses on the IHCs throughout the basal half of the cochlea, and much more slowly, in months to years, as a loss of spiral ganglion cells and their central projections. Therefore, a decrement of amplitude is observed in wave I and not in wave V. The results of present study provide support to the idea that noise induced synaptopathy is selective to low SR fibers, which is indicated by reduction in amplitude at higher compared to lower intensities. A study by Mehraei et al. (2016) wherein, they used noise to find the amount of wave V latency shift, there was decreased wave V latency shift with increase in noise level when compared to the normals. The authors suggested that, the ABR wave V latency shift with noise level is related to the neural desynchronisation, originating from either pre-synaptic event like synaptic vesicle cycle or due to post-synaptic events like

decreased probability of discharge (Liberman, 1978). The low SR ANFs, having higher thresholds, are more resistant to masking by background noise (Costalupes, 1985; Young &Barta, 1986), and as such, their relative contribution to the total neural responses increases as noise level increases. The hypothesis for this can also be related to permanent noise induced threshold shifts. Liberman and Dodds (1984) had also recommended that IHC stereocilia loss, which commonly underlies these irreversible changes, reduces the spontaneous discharge rate of all fibers, presumably because the transduction loss of channels decreases the resting current through the hair bundles, which later slightly depolarises the IHC and decreases spontaneous vesicle release. There could be several possible reasons for the reduction in wave I amplitude and not in wave V. It is an established fact that the generator of wave I is the distal portion of the auditory nerve (Melcher &Kiang, 1996) while wave V is generated at the level of auditory midbrain (Moller et al., 1995; Hall, 2007a). It is because of these different sites of generation, there exists a mechanism between the auditory nerve and the auditory midbrain that might compensate for the reduction in output from the auditory nerve. The hyperactivity in the central auditory pathways was observed in mice with synaptic loss induced by noise exposure (Hickox & Liberman, 2013).

This idea is also supported by studies on tinnitus. Schaette and McAlpine (2001) did a study in individuals with tinnitus and found that the ABR wave I amplitude were reduced in comparison to normal hearing ears without tinnitus, but no differences were reported in wave V amplitude between the two groups. It was concluded by the authors saying the existence of a homeostatic gain control mechanism wherein, they stated that there is an increase in spontaneous firing rate of neurons in the inferior colliculus in the animals subjected to noise exposure. Another

explanation given for the contrasting results of wave I and V amplitude was given by Don and Eggermont (1978). They used a high pass masker to find the frequency contributions to the click evoked ABR. The authors suggest that the generation of wave I is mainly by neurons with characteristic frequencies greater than 2000 Hz whereas, the entire cochlear partition contributes for the generation of wave V. Therefore, it can be postulated that if damage to auditory structures is only present in structures responsible for encoding the higher frequencies in the 3000 Hz to 6000 Hz region commonly affected by NIHL, it is possible that this damage would be revealed as smaller wave I amplitude due to a reduction in the number of neurons contributing to the response. However, this is not the case for wave V amplitude as the structures responsible for encoding lower frequencies remain unaffected and hence, would be unaltered even if the higher frequency neurons are compromised (Don & Eggermont, 1978). These results also explains why ABR wave V/I amplitude ratio is increased in aged and noised exposed ears.

In the present study, it was found that the DPOAEs amplitude was similar in all the three groups examined. There was a significant difference seen only at three frequencies (4358 Hz, 6165 Hz and 6726 Hz) which did not follow any trend. The possible reason for this could be due to damage in the high frequency region, which is in parallel with decrease in ABR wave I amplitude that arises from higher frequency. The reduction in ABR wave I amplitude was evident in all the participants studied suggesting a damage at the synaptic level. Hence, a difference at 4358 Hz, 6165 Hz and 6726 Hz in DPOAEs and for ABR wave I amplitude indicates that the damage at the level of cochlea could be succeeding the damage at the synaptic level.

This result of no decrease in amplitude is line with most of the recent research done on animal ears wherein, the work has shown that there is no change in the DPOAE levels even after full recovery from TTS and there was an absence of permanent noise induced OHC damage in these ears (Kujawa & Liberman, 2009; Lin et al., 2011; Furman et al., 2013). As it is not feasible to conduct anatomical study on OHCs in human subjects, it is in turn assessed on a functional basis like, responses in DPOAEs. Many cross-sectional studies on OAEs have shown that this form of assessment is very sensitive in finding out the damage caused due to noise exposure and ageing without any change in their behavioural thresholds (Attias et al., 2011).In individuals having their behavioral thresholds within normal range (typically defined as $\leq$ 20 dB nHL), there is no strong support from the literature as well for the use of DPOAEs in detecting earlier damage to the auditory system (Lapsley Miller, Marshall, Heller, & Hughes, 2006; Marshall et al., 2009; Seixas et al., 2012).The major damage in ageing and occupational noise exposed individuals occurs at a high frequency region (Attias et al., 2001).

Therefore, it is difficult to identify an individual of his damage due to ageing and noise exposure at an earlier stage using DPOAEs as a measure. Thus, we can infer that, ABR wave I amplitude reduction and increment in wave V/I amplitude ratio (which is due to the lessening of wave I amplitude and not because of wave V changes), acts as former clinical indicators when compared to DPOAEs suggesting prior to hair cell damage there is damage at the synaptic level.

Chapter 6

Summary and Conclusion

Regular exposure of cochlear amplifiers to high level noise and changes as a part of ageing in humans may yield irreversible damage to them. The role of the efferent system presumably is to enhance signals in the presence of noise and, an ideal test used for identifying the shifts observed in cochlear functioning would be OAEs. OAEs are preferred over pure tone audiometry for early identification of NIHL because they are sensitive to minor damage to outer hair cells and also can be monitored easily due to their objectivity and speed. However, in early stages, there may not be any evident threshold shift even in the presence of underlying efferent system damage. Previous studies have reported neural degeneration in ears with noise-induced threshold shifts and ageing, suggesting that normal hearing thresholds can be accompanied by impaired function of efferent fibers that project from the brainstem to the cochlea. Hence, assessment at the brainstem level provides valuable information on early identification of such conditions.

Hence, this study was taken up to compare the functioning of auditory system in ears of ageing and occupational noise exposure and to find a better marker for early identification of these conditions. Thirty adult males were divided into three groups of ten individuals each. Individuals aged >45 years without any occupational noise exposure formed Group 2 (N=10) and individuals who are exposed to noise greater than 80 dB(A) for a duration of 8 hours per day in their workplace with age <35 years constituted Group 3, with Group 1 being the control group. DPOAE fine structure was studied at 8 points per octave with f1 and f2 primaries being presented at 65 dB SPL and 55 dB SPL respectively at different frequencies. ABR was recorded using clicks

at five intensities (90, 80, 70, 60 & 50 dBnHL) having a repetition rate of 7.1/sec through ER-3A Insert phones in Interacoustics Eclipse EP-25 in aged and occupational noise exposed individuals along with a control group. The results indicated a significant difference in the amplitude of wave I in which, the amplitude was reduced in Group 2 and 3 compared to Group 1. There was no difference in the amplitude of wave V at any of the tested frequencies which could be because of the homeostatic gain control mechanism wherein, there is an increase in spontaneous firing rate of neurons in the inferior colliculus. The DPOAEs amplitude did not show any significant difference at almost all the tested frequencies suggesting no damage at the level of OHCs. Hence, from the present study we can conclude that ABR wave I amplitude and increase in wave V/I amplitude ratio (which is due to the lessening of wave I amplitude and not because of wave V changes), acts as a reliable marker in the early identification of damage to ageing and noise exposed ears among all the parameters studied.

6.1. Implications of the study

1. The use of supra-threshold stimuli in ABR provides an evidence of early-onset noise-induced auditory damage that is not obvious in the routine test like threshold assessment, as the current gold standard for NIHL assessment is based on threshold determination and absence of OAEs.

2. Early identification of noise-induced auditory damage helps in preventing further damage to the auditory structures and also to minimise the effects of hearing loss on the individual.

3. Counselling about ear protective devices at an early stage reduces the extent of hearing loss, which might enhance due to combined effects of aging and noise exposure at a later stage.

6.2. Future directions

1. To carry out the study with larger number of samples, for better generalization of results.

2. To verify whether similar results are obtained when the same measures are analysed at various noise exposure durations and across different age groups.

3. To compare these results with behavioral tests assessing temporal coding ability.

6.3. Limitations of the study

1. The number of participants in the study was limited to 30, to generalize the findings a larger sample size would have been appropriate.

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Appendix 1

The MANOVA test results for frequencies that exhibited no significant difference in Group 1, Group 2 and Group 3

Frequency (Hz)	F value
842	$F(2, 48) = 0.01$, $p > 0.05$
916	$F(2, 48) = 1.96$, $p > 0.05$
1001	$F(2, 48) = 0.03$, $p > 0.05$
1086	$F(2, 48) = 1.66$, $p > 0.05$
1184	$F(2, 48) = 1.75$, $p > 0.05$
1294	$F(2, 48) = 1.53$, $p > 0.05$
1416	$F(2, 48) = 0.54$, $p > 0.05$
1538	$F(2, 48) = 2.53$, $p > 0.05$
1685	$F(2, 48) = 1.57$, $p > 0.05$
1831	$F(2, 48) = 0.81$, $p > 0.05$
2002	$F(2, 48) = 0.24$, $p > 0.05$
2185	$F(2, 48) = 0.26$, $p > 0.05$
2380	$F(2, 48) = 1.99$, $p > 0.05$
2600	$F(2, 48) = 0.32$, $p > 0.05$
2832	$F(2, 48) = 1.51$, $p > 0.05$
3088	$F(2, 48) = 3.24$, $p > 0.05$
3369	$F(2, 48) = 3.35$, $p > 0.05$
3662	$F(2, 48) = 2.30$, $p > 0.05$
4004	$F(2, 48) = 0.47$, $p > 0.05$
4761	$F(2, 48) = 0.29$, $p > 0.05$
5188	$F(2, 48) = 0.91$, $p > 0.05$
5652	$F(2, 48) = 0.58$, $p > 0.05$
7336	$F(2, 48) = 1.30$, $p > 0.05$
7996	$F(2, 48) = 1.67$, $p > 0.05$