Effect of Varying the Interstimulus Interval on Multi Frequency Auditory Brainstem Response

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CERTIFICATE

This is to certify that this dissertation entitled **'Effect of Varying the Interstimulus Interval on Multi Frequency Auditory Brainstem Response'** is the bonafide work submitted in part fulfillment for the degree of Master of science (Audiology) of the student (Registration No: 16AUD012). 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 or any other diploma or degree.

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CERTIFICATE

This is to certify that this dissertation entitled **'Effect of Varying the Interstimulus Interval on Multi Frequency Auditory Brainstem Response'** has been prepared under my supervision and guidance. It is also certified that this has not been submitted earlier to any other university for the award or any other Diploma or Degree.

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DECLARATION

I hereby declare that this dissertation entitled **'Effect of Varying the Interstimulus Interval on Multi Frequency Auditory Brainstem Response'** is the result of my own study under the guidance of **Dr. Sandeep M**, Reader in Audiology, Department of Audiology, All India Institute of Speech and Hearing, Manasagangothri, Mysore and has not been submitted earlier to any other university for the award or any other Diploma or Degree.

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Dedicated

to my

Mom - Dad,

Brother,Atap, my

Guide and JC.

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Chapter 1

INTRODUCTION

Auditory brainstem responses (ABRs) are clinically used to evaluate the peripheral auditory system and the lower brainstem. As its primary clinical application, ABRs are used to estimate hearing thresholds in patients who are not able to provide reliable behavioural thresholds such as infants and malingering adults. (Coles, 1977; Hall, 1992; Stapells & Vancouver, 2000). The information derived from ABR is useful for inferring the degree of hearing loss, configuration of hearing loss, type of hearing loss and to an extent, the cause of hearing loss. This information in turn helps in fitting of hearing aids, facilitating early identification and rehabilitation (Hoke, Pantev, Ansa, Lutkenhoner & Herrmann, 1991). However, there are still limitations to ABR testing, not the least of which is the time duration of a test session particularly while estimating frequency specific ABR thresholds (Mitchell & Clemis, 1977; Jerger et al., 1985; Burkard et al., 1990; Hamill et al., 1991; Mitchell et al., 1994).

The testing duration of ABR is governed by three major factors; the number of averages required for an acceptable signal to noise ratio, the repetition rate of the stimulus and the number of frequencies for which the threshold is to be estimated. These three factors are particularly important in frequency-specific ABR testing, where the time required to obtain thresholds to a comprehensive number of stimulus frequencies usually exceeds the time which is available by patient sedation or cooperation (Mitchell & Clemis, 1977; Davis et al., 1985).

Reducing the test time by increasing the repetition rate is limited by neural adaptation, which degrades the ABR morphology (Smith & Brachman, 1982). Particularly, presentation rates above 20/s (Fowler & Noffsinger, 1983; Campbell & Abbas, 1987), typically results in diminished ABR amplitudes (Leung, Slaven, Terkildsen & Osterhammel, 1975) and longer ABR latencies (Stapells & Picton, 1981; Leunget al., 1998). Because some reduction in wave V amplitudes is tolerated, adapted rates of 25–40/s are acceptable in threshold testing (American Speech Language-Hearing Association, 1987). However, rates below 25/s are advisable to ensure clear ABR morphology, more so in neuro-diagnostic evaluations (American Speech-Language-Hearing Association, 1987; Hall, 2004) and paediatric population.

To avoid the adaptation effects caused by increasing the stimulus presentation rate in frequency specific ABR testing, some researchers have trailed alternative stimuli called 'chained stimuli'. The tone bursts of different frequencies are chained one after the other with appropriate inter-stimulus interval to generate a chained stimulus. Instead of eliciting ABRs for tone bursts individually with high repetition rate, a chained stimulus involving all tone bursts in one recording can be used with lower repetition rate without causing adaptation. This approach interleaves several discrete stimuli and maximizes acquisition efficiency, while minimizing response adaptation. It is assumed that if the frequency of each discrete stimulus is different enough, then different populations of neurons will be stimulated in sequence, and adaptation will be minimized or avoided even if the inter-stimulus interval is reduced to as low as 10 milliseconds (Mitchell, Fausti & Frey, 1994; Mitchell, Henry, Kempton, Fausti & Trune, 1994). The conventional method used to obtain frequency specific ABR is to stimulate the auditory system with brief tone bursts with short rise times (Suzuki & Horiuchi, 1977; Klein & Teas, 1978; Kodera, Yamada, Yamane & Suzuki, 1978). This approach is limited, however, by its excessively long test time, approximately 2 hours (Stueve & O'Rourke, 2003; Karzon & Lieu, 2006).

Mamatha (2016) estimated ABRs using multifrequency chain of tone bursts and compared it with that of single frequency ABR. The results showed that multi frequency auditory brainstem response (MFABR) had comparable latency, amplitude and thresholds, and therefore would not affect clinical interpretation.

1.1 Justification for the Study

To estimate hearing thresholds in difficult to test population, where in behavioural thresholds are not reliable, objective techniques such as click ABRs can be used. Click evoked ABRs predominantly estimates hearing between 1000Hz and 4000Hz (Emanuel, 2002) but these estimates are not frequency specific. However, it is one of the most preferred techniques due to its time efficiency.

Frequency specific auditory thresholds are vital for fitting hearing aids. Auditory evoked potentials such as tone burst ABR, Auditory steady state responses (ASSR) and Late latency responses (LLR) are known to be reliable techniques for estimating frequency specific hearing thresholds. However, these test procedures are not practiced in all clinical set ups due to time constraints. Approximately 2 hours are required for tone burst auditory brainstem response (Stueve & O'Rourke, 2003; Karzon & Lieu, 2006), around 28 minutes for MASTER ASSR (Schmulian, Swanpoel & Hugo, 2005) and approximately 4 hours for LLR (Bell, Smith, Allen & Lutman, 2004) has been estimated.

The estimated testing duration for MFABR on the other hand is 30 minutes according to Mamatha (2016). Although ASSRs are quicker in acquisition of frequency specific auditory thresholds, they are highly contaminated by stimulus related artifacts resulting in high false positives (Gorga et al., 2004; Picton & John, 2004; Small & Stapelles, 2004). Even though it is possible to obtain frequency specific thresholds with the LLR, its susceptibility to the state of arousal, drugs and longer test duration curtails their usefulness. In such conditions, ABR can be obtained for tone bursts using chained stimuli of multiple frequencies.

There are only fewer studies assessing acquisition of ABR with multiple frequency and multiple intensity tone bursts (Mitchell, Fausti & Frey, 1994; Mitchell, Kempton, Creedon & Trune, 1996; Curtin, Mitchell, Kempton, Creedon & Trune, 1999). One set of studies (Mitchell et al., 1996; Mitchell et al., 1999) are done on mice at frequencies above 8000Hz and the results obtained cannot be directly generalized to human population. In the study by Mitchell, Fausti & Frey (1994) on humans, they did not use stimulus frequencies below 8kHz, which limits the applicablity of the results to study hearing thresholds in human beings.

Mamatha (2016) recorded ABR using a chain of 4 tone bursts with onset to onset interval of 20ms. The frequencies used were 500Hz, 1000Hz, 2000Hz and 4000Hz. Results supported the use of MFABR as a time efficient clinical tool to estimate frequency specific hearing sensitivity within 20 minutes. However, the onset to onset interval of 20ms allows the tester to use only lower repetition rate of less than 10/second. If reducing the interval between tone bursts does not affect the response, it allows the tester to use higher repetition rate, in turn cutting down the testing time. Considering that different group of neurons are involved in different tone bursts, it shall have minimal effect. To systematically study the effect of varying onset to onset interval of tone bursts on MFABR, the present study was taken up.

1.2 Aim of the Study

The aim of the study was to investigate the effects of varying Interstimulus Interval (ISI) on multifrequency ABR (MFABR).

1.3 Objectives of the Study

- **i.** To compare latency of ABR elicited by MFTBs with an ISI of 20 ms with that of ABRs elicited by MFTB with an ISI of 15 and 10 ms at 90dBnHL
- **ii.** To compare amplitude of ABR elicited by MFTBs with an ISI of 20 ms with that of ABRs elicited by MFTB with an ISI of 15 and 10 ms at 90dBnHL
- **iii.** To compare latency of ABR elicited by MFTBs with an ISI of 20 ms with that of ABRs elicited by MFTB with an ISI of 15 and 10 ms at 40dBnHL
- **iv.** To compare amplitude of ABR elicited by MFTBs with an ISI of 20 ms with that of ABRs elicited by MFTB with an ISI of 15 and 10 ms at 40dBnHL

1.4 Hypothesis

The null hypothesis of the present study was that there is no significant difference in the latency and amplitude of MFABRs elicited across the 3 ISIs at 90 and 40dBnHL.

Chapter 2

REVIEW OF LITERATURE

Frequency specific auditory brainstem responses (ABRs) have been recorded using a plethora of stimuli. Initial research mainly focussed on the use of tonal stimuli/tone bursts for the same. This became the convention whenever frequency specific information was required, as the results obtained were reliable as well as valid. Later on, with the advent of technology, many new techniques were proposed for obtaining frequency specific ABRs. This included the use of new stimuli or the use of high pass or puretone maskers, use of a chain stimuli and derived band.

2.1 Conventional Methods used to Obtain Frequency Specific ABR

The conventional method makes use of frequency specific tone bursts to which auditory brainstem responses are recorded. It involves the use of brief tone bursts of short rise times which stimulates the auditory system to obtain frequency specific information (Suzuki & Horiuchi, 1977; Klein & Teas, 1978; Kodera, Yamada, Yamane & Suzuki, 1978). This usefulness of this method is however curtailed by its excessively long testing time of approximately 2 hours (Karzon & Lieu, 2006; Stueve & O'Rourke, 2003).

Stapells, Picton, Perez-Abalo, Read and Durieux-Smith (1985) conducted a meta-analysis of 32 studies consisting of a total of 1,203 participants (i.e., 524 adults, 679 infants; 815 individuals with normal hearing; and 388 individuals with SNHL). Results indicated that ABR thresholds in individuals with normal hearing were 10 to 20 dB nHL thereby corelating well with behavioural puretone thresholds. Auditory brainstem response thresholds in participants with SNHL were found to be 5 to 15 dB higher than pure-tone behavioural thresholds in adult participants and 10 dB lower to 10 dB higher in infants and young children.

Gorga, Kaminski, Beauchaine and Jesteadt (1988) recorded auditory brainstem responses from 20 normal hearing individuals in response to tone burst stimuli. Results revealed the ABR thresholds to be higher than the behavioural audiometric thresholds for all the frequencies with a marked difference and greater inter-subject variability seen for lower frequencies such as 250Hz and 500Hz.

Studies have reported similar findings in bone conducted tone burst ABRs (Boezeman, Kapteyn, Visser & Snel, 1983; Foxe & Stapells, 1993; Kramer, 1992; Stapells & Ruben, 1989). Better responses at higher frequencies were attributed to shorter rise times of the tone burst as they result in greater discharge synchrony, which in turn results in greater amplitude of the response relative to the background noise. As compared to the apical region, the basal end of the cochlea has a greater nerve fibre density (Spoendlin, 1972). This increased density results in a greater number of neural fibres discharging synchronously for high frequency stimuli.

Dündar et al. (2014) compared thresholds of tone-burst auditory brainstem responses and puretone audiometry on eighty individuals with sensorineural hearing loss at 500Hz, 2000Hz and 4000Hz, and the differences between tone-burst auditory brainstem response thresholds and pure-tone thresholds were calculated. The mean difference between thresholds of tone-burst, and pure-tone audiometry was found to be 4.75dB, 6.25dB, and 4.87dB at 500Hz, 2000Hz and 4000Hz respectively.

Suzuki, Kodera and Kaga (1982) compared auditory brainstem responses and behavioural thresholds at 500Hz and 1000Hz. They reported auditory brainstem responses thresholds to be higher than behavioural thresholds. Hayes, Jerger and Jerger (1982) reported that there is an inherent difference in our ability to elicit an auditory brainstem response for lower frequencies. The greater variability in the differences between auditory brainstem responses and behavioural thresholds for lower frequencies may be the limiting factor in using tone-burst ABRs to predict behavioural thresholds. However, utility of tone burst auditory brainstem response to obtain frequency specific responses for all frequencies is limited by its excessively long test time of approximately 2 hours (Stueve & O'Rourke, 2003; Karzon & Lieu, 2006).

2.2 Non-conventional Methods used to Obtain Frequency Specific ABRs

The two major non-conventional methods used to obtain auditory brainstem responses include the masking method and the derived band method. The masking method employs the use of a masker in order to eliminate unwanted non-frequency-specific contributions to the auditory brainstem response by selectively masking cochlear regions which are outside the region to be stimulated either by using notched-noise masking or high-pass masking noise (Terkildsen, 1975; Picton, 1979; Stapells & Picton, 1981; Pratt & Bleich, 1982; Jacobson, 1983; Stapells, 1990; Beattie & Kennedy, 1992; Beattie, 1992; Conijn, 1992; Abdala & Folsom, 1995; Oates & Stapells, 1997).

Alternately, the neural activity in specified cochlear regions can also be selectively suppressed by computing the off-line difference-waveform between the masked and unmasked responses by using derived response technique (Eggermont, 1976;

Don & Eggermont, 1978; Eggermont & Don, 1980; Kramer, 1992; Nousak & Stapells, 1992; Donaldson & Ruth, 1993; Don, 1994, 1997; Oates & Stapells, 1997) or by using pure-tone masking methods (Folsom, 1984, 1985; Pantev, 1985; Klein, 1983; Klein & Mills, 1981; Mackersie, 1993; Wu & Stapells, 1994).

2.2.1 Masking Methods used in ABR

Picton, Ouellette, Hamel and Smith (1979) suggested the use of notched noise as it can be used to mask the frequency spread of acoustic energy thereby providing more frequency specific responses.

Stapells and Picton (1981) recorded tone bursts auditory brainstem responses at 0.5 kHz and 2 kHz with and without notched noise in 11 normal hearing subjects in the age range of 22 to 30 years. The notched noise reduced the amplitude of the response particularly at high intensities. They reasoned that the notched noise limits the auditory brainstem response to a particular area of the cochlea, effectively masking out the responses that are mediated through other regions of the cochlea due to the spread of acoustic energy of the stimuli or by the dynamics of the travelling wave. Hence, they concluded that the responses at and above 100 dB is less frequency specific and recommended the used of notched noise method especially at high intensity levels.

Beattie and Spence (1991) used notched noise method to estimate frequency specific responses. They reported that high noise levels are essential to mask clicks (95dB SPL of BBN is required to mask a 65 dB nHL click) which in turn would lead to tolerance issues. High ABR thresholds were obtained in notched noise method. Based on their results they suggested tone bursts to be more promising stimuli for assessing the frequency specific auditory brainstem responses than the notched noise method. A similar finding was reported by Orsini (2004).

High pass masking noise method is also used to increase frequency specificity in auditory brainstem responses (Don & Eggermont, 1978; Kileny, 1981; Laukli, 1983; Stapells et al., 1985). High pass noise masking method is reported to provide larger amplitudes than notched noise, thus leading to a greater response identifiability. Also, it requires less complex instrumentation than notch noise. However, tone bursts in high pass noise are not as frequency specific as tone bursts in notched noise because the stimulus includes all frequencies below the cut-off frequency. However, for frequencies below 1000Hz it can still serve to be a good tool to elicit frequency specific information.

Don and Eggermont (1978) proposed a novel technique called derived band technique to obtain frequency specific auditory brainstem responses in which auditory brainstem responses are first recorded for clicks alone and then with various high pass noises having different cut off frequencies. An offline subtraction of 2 auditory brainstem responses elicited with high pass noise of 2 adjacent cut off frequencies will give derived band auditory brainstem responses. The assumption of the technique is that these responses differ only in the contribution of the frequency region between the cut-off frequencies of the maskers. Therefore, the subtracted response originates from a limited frequency region only. Although this assumption seems true, and the technique has been experimentally validated, it is again time consuming, needs computer storage, and because of the subtraction of two responses, signal to noise ratio of the responses decrease. Furthermore, contribution from the region below 500Hz for auditory brainstem response elicited by clicks is probably minimal (Don & Eggermont, 1978; Don et al., 1979; Thiimmler et al., 1981; Laukli et al., 1988; Gorga et al., 1988) and therefore may not elicit identifiable responses in the 500Hz band.

2.2.2 Use of Multi-frequency ABR (MFABR) in Eliciting Frequency Specific ABRs

Mitchell, Fausti and Frey (1994) were amongst the first researchers to make use of a series of tone bursts to elicit frequency specific information. Stimuli comprised of 21 tone bursts ranging from 1kHz to 32kHz in 1/4-octave steps. The duration of these tone bursts was 2ms, with a rise/fall time of 1ms and no plateau. Factors such as the intrapair time, frequency, and intensity were varied in order to determine the onset of adaptation which is characterised by a latency delay. Results showed that the adaptation effects are minimal when the time separation is 10ms or greater in paired-stimulus. Further, adaptation was reported to be generally less if the frequency of the second stimulus was either above or below that of the first stimulus in paired stimulus. This study was conducted on guinea pigs and hence can't be generalized to the human population.

Fausti, Mitchell, Frey, Henry and O'Connor (1994) recorded auditory brainstem responses for high-frequency tone bursts in two different methods in a single session. Ten normal hearing subjects participated in the study. Step 1 involved presentation of four high-frequency tone burst stimuli (14kHz, 12kHz, 10kHz and 8kHz) individually while Step 2 involved presentation of multiple stimulus sequence with a variable onset to onset interval ranging from 3 to 30ms. Wave V latencies were compared for these test conditions. Results showed that there was a small but statistically insignificant prolongation in latency observed for the multiple stimulus sequence when the ISI was 10ms or higher with a good Test-retest reliability. These findings corroborate the use of chain stimuli in clinical settings as a tool for obtaining frequency specific information within a lesser time duration. The drawback of this study was that it was done for frequencies above 8kHz which limits its applicability.

Mitchell, Kempton, Creedon and Trune (1996) recorded ABRs for a chain of tone bursts ranging from 4kHz to 32kHz and having a 12ms inter-stimulus interval. The chain consisted of 20 tone burst sequences of four different frequencies. The results indicated no significant differences in thresholds or waveform characteristics when compared to single tone burst recordings.

Mitchell, Kempton, Creedon and Trun (1999) found similar results in mice while using a chain of 56 stimuli train spaced at 0.5 octave from 4 to 32 kHz. These studies, however, were carried out on mice hence limiting its generalisation to the human population again.

Petoe, Bradley, and Wilson (2009) made use of a series of chained tone – pulse stimulations in the presence of a gliding high pass noise (GHINOMA) in order to compare the latency of Wave V obtained to those for ABRs evoked by conventional tone bursts. The results obtained validated the use of chained stimuli as an effective and less time consuming clinical tool for obtaining frequency specific ABR's.

Mamatha (2016) recorded ABRs using a chain stimulus consisting of 4 tone bursts of 4kHz, 2kHz, 1kHz and 0.5kHz having a 2-0-2 cycle and an onset to onset interval of 20ms. Participants consisted of 30 normal hearing individuals and 11 individuals with sensorineural hearing loss in the age range of 20 to 50 years. The latency and amplitude of wave I, III and V were compared between conventional single frequency tone burst ABR and the MFABR recordings. They reported no significant differences between the MFABR and conventional single frequency tone burst recordings. Moreover, it correlated well with the behavioural audiometric thresholds at the respective frequencies thereby establishing MFABR as a reliable and less time-consuming tool for clinical ABR recordings as the entire test procedure requires around 30 minutes to complete.

Swathy (2017) recorded MFABR in infants using the same protocol as that of Mamatha (2016). MFABR and conventional single frequency tone burst ABRs along with Behavioural Observation Audiometry was recorded from 21 infants in the age range of one month to one year. No significant differences between the MFABR and Behavioural Observation Audiometry responses were reported. The MFABR and single frequency tone bursts were also in agreement with each other.

Overall, literature reveals that tone bursts elicit reliable frequency specific ABRs and the thresholds obtained are in close agreement with the behavioural thresholds. This is true in in both adults and infants, in air conduction as well as bone conduction modality. Studies in Multifrequesncy ABRs show a strong evidence for the reliable recording of frequency specific ABRs with remarkable time efficiency. However, further studies are warranted to probe the other possible methods of cutting down the testing time within MFABRs.

Chapter 3

METHOD

The current study aimed to investigate the effects of varying the interstimulus interval on Multi Frequency Auditory Brainstem Responses (MFABR). The null hypothesis was that there is no significant difference in the latency and amplitude of MFABR elicited across the 3 ISIs used. The following procedure was used to test the hypothesis.

3.1 Participants

Twenty adults in the age range of 18 to 25 years participated in the study. It was ensured that the participants had normal hearing sensitivity (Puretone hearing thresholds within 15dBHL) at octave frequencies between 250 to 8000 Hz and normal middle ear functioning (Type 'A' tympanogram with acoustic reflexes present). A detailed case history was taken to ensure that they did not have any relevant otological or neurological dysfunction. Screening test for Auditory processing (STAP) (Yathiraj & Maggu, 2012) was administered to screen out Auditory Processing Disorders. The presence of click evoked ABRs recorded using a protocol appropriate for site of lesion testing ensured that the auditory nerve and brainstem functioning was normal. An informed consent was obtained from each participant prior to their participation.

3.2 Instrumentation

A calibrated diagnostic audiometer Grason-Stadler Inc – 61 (with TDH-39 supra aural headphones) was used for pure tone audiometry and speech audiometry. Grason-

Stadler Inc -Tympstar middle ear analyser was used to record tympanograms and acoustic reflex thresholds. A Biologic Navigator Pro auditory evoked potential system with impedance matched insert receiver was used for acquiring ABRs.

3.3 Test Stimulus

The stimuli consisted of four tone bursts (TBs); 4000, 2000, 1000 and 500 Hz, chained one after the other in the same sequence as mentioned. They were generated using Praat software (version 5.3.36) with 2-0-2 envelope and Hanning window. Accordingly, the duration of the stimuli for 4000, 2000, 1000 and 500 Hz were 1ms, 2ms, 4ms and 8ms respectively.

The tones bursts were objectively calibrated to ensure equal output SPL across them. The output SPL of each of the four TBs was recorded using an SLM (Bruel & Kjaer with Pressure-field 1" microphone type 4144) using standard settings. Tone bursts were routed through ER3A insert receivers connected to the Biologic Navigator Pro EP system and were played at 110dB SPL. The amplitudes of the generated TBs were then manipulated such that the peak SPL measured was 110dB SPL in each of the frequencies.

The individual TBs were then played to 20 normal hearing individuals to obtain the minimum dB SPL required to hear them. For this, the TBs were routed through the same insert receivers and were presented at the rate of 9.1/s. The average of the threshold of 20 individuals for tone burst of each frequency was calculated. This value served as a correction factor and was used to derive ABR threshold in terms of nHL.

The calibrated TBs were then chained together in the order of 4000, 2000, 1000 and 500 Hz. Three such stimuli chains were generated, one each with an ISI of 20, 15 and 10ms. The corresponding total duration was 68, 53 and 38ms for the 20, 15 and 10 ISI chain respectively. Figure 3.1 shows the waveform of the 3 TB chains used in the present study. Figure 3.2 shows the spectrum of the same 3 TB chains.

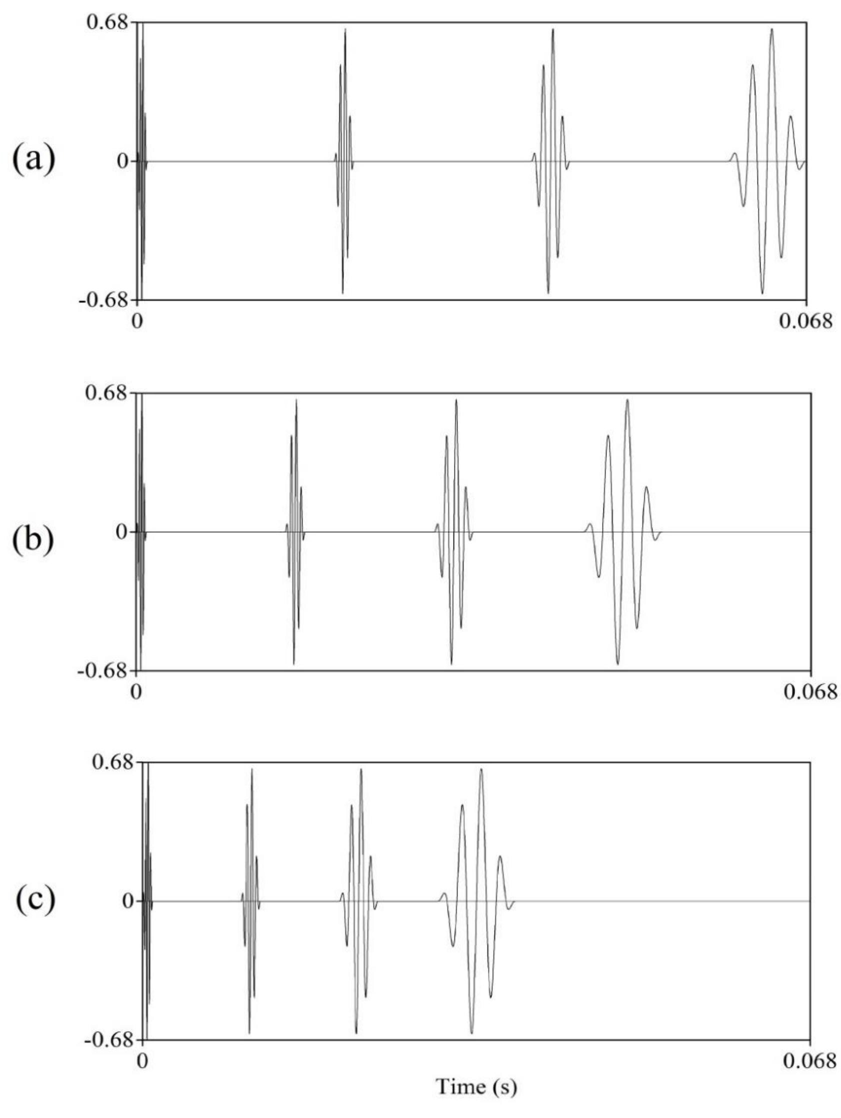

Figure 3.1: Waveform of the multifrequency chain of tone bursts with (a) 20ms ISI, (b) 15ms ISI and (c) 10ms ISI, used in the present study.

3.4 Test Environment

All preliminary tests were carried out in a sound treated room where the noise levels were within permissible limits (ANSI S-3, 1991). The ABR recordings were carried out in an electrically shielded room (Electrophysiology Lab, Dept of Audiology, AIISH) where the noise levels were below the permissible limits

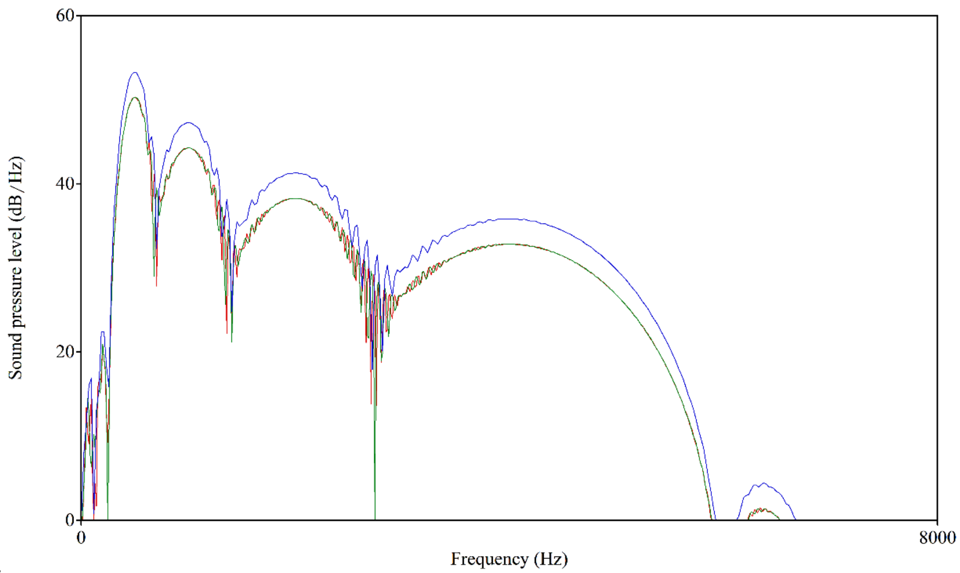

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Figure 3.2: Spectrum of the 3 multi frequency chain of tone bursts wherein the red, green and blue lines represent chains with 20, 15 and 10 ISI respectively.

3.5 Test Procedure

The test procedure involved preliminary audiological evaluations to assess the candidacy of the prospective participants. Once the candidacy was ensured, they were subjected to the experimental procedure which included recording of MFABR.

3.5.1 Preliminary Audiological Evaluations

Modified Hughson and Westlake procedure (Carhart & Jerger, 1959) was used to estimate the *pure tone hearing thresholds*. Thresholds were estimated at octave frequencies between 250Hz and 8000Hz for air conduction stimulation.

Immittance evaluation involved recording tympanograms and acoustic reflexes. A 226 Hz probe tone at approximately 85dBSPL was used to obtain the tympanograms. The air pressure in the ear canal was varied from +200 to -400 daPa. Static admittance and peak pressure were recorded in order to interpret the audiogram and thereby rule out middle ear pathology. Ipsilateral and contralateral acoustic reflex thresholds were measured for 500, 1000, 2000, and 4000 Hz pure tones using probe tone of 226 Hz.

Click evoked ABRs were recorded to check for the integrity of the neural pathway at the level of brainstem prior to MFABR and also to rule out brainstem lesions. Only if the results of click ABR were normal, the individual was considered to record MFABR. The conventional settings and parameters recommended for site of lesion testing in ABR (Hall, 2007) were used for recording click ABRs.

3.5.2 Recording MFABR

The participants were made to sit on a reclining chair and were instructed to relax and minimize extraneous movements. The surface electrode sites (Cz, M1 & M2) were cleaned before placing electrodes and inter electrode impedance was maintained below 2 kOhms. Three silver chloride disc electrodes were placed in a vertical montage with Cz being positive, M2 being negative and M1 being the ground electrode sites. Three sets of

ABRs were recorded for each participant; one set each for stimulus of 20, 15 and 10ms ISI. For the stimulus with 20ms ISI, responses were recorded at 90, 60 and 40dBnHL. Whereas for the stimulus with other two ISIs, responses were recorded only at 90 and 40dBnHL. Table 3.1 shows the stimulus and acquisition parameters used for recording MFABR.

3.6 Response Analysis

Only if the waves were replicable, they were considered for response analysis. The averaged ABRs were visually analysed to mark the presence of Jewett waves, I, III and V. The responses were independently analysed by 3 audiologists, experienced in the area of electrophysiology. The parameters considered for marking the peaks were: the replicability of the recorded waveforms, negative slopes, and latency characteristics of the peaks. The recorded waveforms were overlapped after which the peaks were marked. The peak latency and peak amplitude of the waves present were noted down from each set.

Table 3.1: Stimulus and acquisition parameters used for recording MFABR

Stimulus Parameters	
Stimuli	Multi - frequency chain of tone bursts of 20, 15 and 10 (onset to onset) ISI
Duration	68, 53 and 38ms respectively
Polarity	Rarefaction
Repetition Rate	9.1Hz
Intensity	For 20 ISI stimuli: 90, 60 & 40dBnHL For 15 & 10 ISI: 90 & 40dBnHL
Ear of Stimulation	Right ear

Acquisition Parameters	
Montage	Vertical
Electrode sites	Cz (+ve) M2 (-ve) M1 (Reference)
Filters setting	100 – 1500 Hz
Amplification	1,00,000
Artifact Rejection	19µV
Analysis time	85ms
Total no. of averages	2000
Data points	1024

Chapter 4

RESULTS

The objective of this study was to test whether there is any significant difference in results of multifrequency auditory brainstem responses (MFABR) when the interstimulus interval (ISI) is varied. The MFABRs were recorded at interstimulus intervals of 20, 15 and 10ms. A total of 168 parameters were tested for normality which included the latency and amplitude of I, III and V waves, at ISIs of 20, 15 and 10ms, in different intensities. The group data was tested for its distribution using Shapiro-Wilk test of normality. The results revealed that the data did not follow normal distribution and hence Wilcoxon sign rank test was used to compare across the conditions. The results of the study are reported under the following sections

1. Comparison of MFABR across intensities
2. Comparison of MFABR across the three multifrequency chains of tone bursts of different ISIs

4.1 Comparison of MFABR Across Intensities

MFABRs were recorded at 90, 60 and 40 dBnHL and the data obtained was compared on the basis of prevalence, latency and amplitude of waves I, III and V at four different frequencies (4000Hz, 2000Hz, 1000Hz & 500Hz). This was done only for MFABRs recorded for stimulus chain of 20ms ISI. Figure 4.1 shows a set of MFABRs recorded at 90, 60 and 40dBnHL in a representative participant. In the stimulus chain with 20 ms ISI, tone bursts (4000Hz, 2000Hz, 1000Hz & 500Hz) the subsequent tone bursts started after every 20 ms. Therefore, one ABR was generated after every 20 ms. In the figure, one can see four ABRs, on each generated for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz. Furthermore, as the intensity decreased, there was prolongation of latencies and generally, decrease in the amplitude.

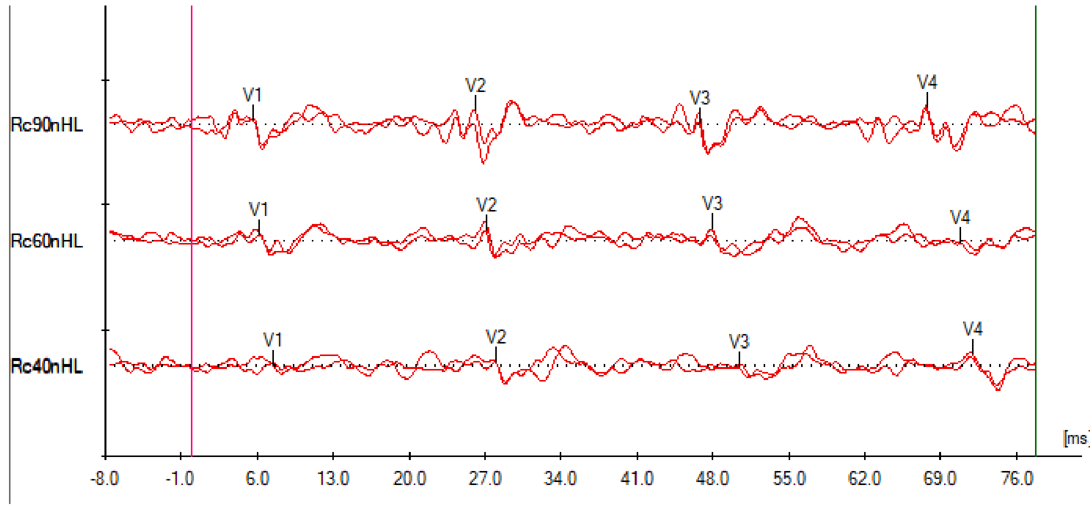

Figure 4.1: MFABRs at 90, 60 and 40 dBnHL recorded in a representative participant for stimulus of 20ms ISI.

4.1.1 Results of Prevalence of ABR Waves

Prevalence of ABR wave was operationally defined as the number of participants who had responses present, out of the total number of participants (20), and is expressed in percentage. The prevalence of waves seen for stimulus chain of 20 ms ISI, for the tone bursts of four frequencies (4000Hz, 2000Hz, 1000Hz & 500Hz) across different intensities is shown in Figure 4.2. The prevalence was highest at 90dBnHL for all the three waves (I, III & V) and reduced as the intensity decreased. Wave V was present in most of the participants .

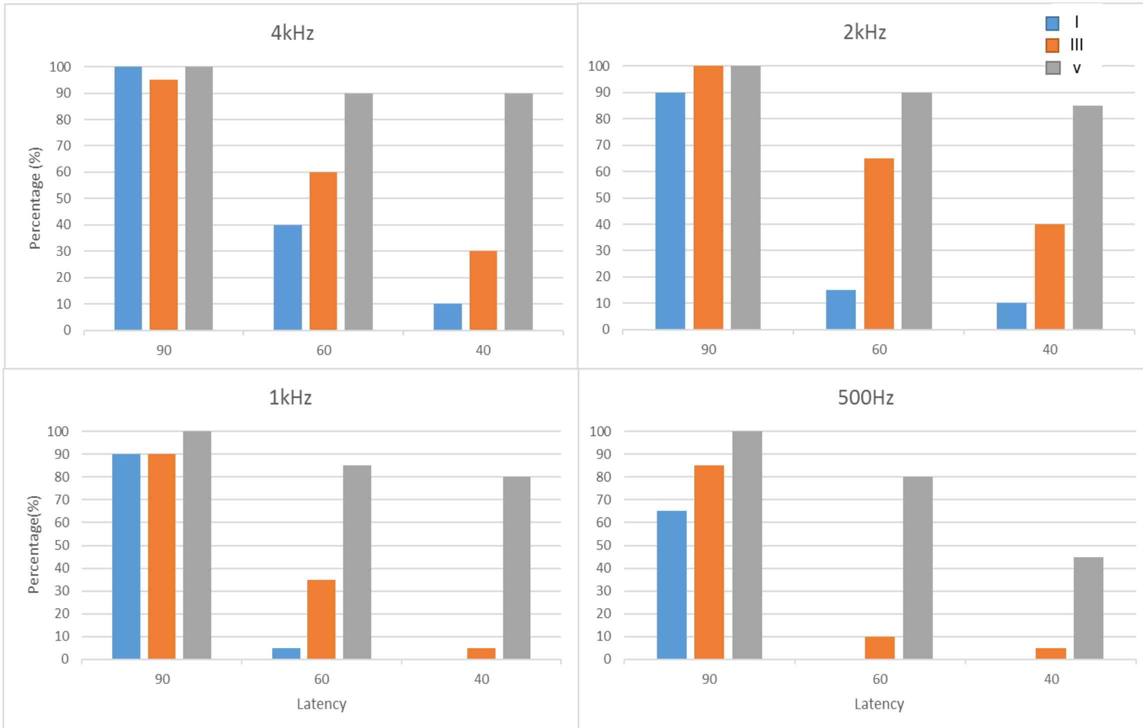

Figure 4.2: Prevalence of wave I, III and V across 90, 60 and 40 dBnHL for 20 ms ISI recroded at the four test frequencies in MFABR.

Results of Peak Latency

Table 4.1 gives the median and standard deviation of peak latency of wave I, III and V. Figure 4.2 shows the mean latencies of the three waves across intensities at four different frequencies, elicited using MFABR. On comparison, it was observed that the latency of the waves increased as the intensity decreased. This trend was seen at all frequencies. When comparing across frequencies, an increase in the latency of peaks as the frequency of the tone burst decreased was a general trend that was observed.

Table 4.1: Median and Standard deviation of peak latency (ms) of waves I, III and V at four different frequencies (4000Hz, 2000Hz, 1000Hz & 500Hz) at three intensities (90, 60 & 40 dBnHL), elicited using MFABR

Wave	Frequency of TB	90dBnHL		60dBnHL		40dBnHL	
		Median	SD	Median	SD	Median	SD
I	4kHz	1.87	0.1	2.32	0.16	2.65	0.23
	2kHz	2.32	0.21	3.16	0.54	4.03	0.53
	1kHz	2.74	0.25	4.49	*	*	*
	500Hz	2.99	0.58	*	*	*	*
III	4kHz	3.82	0.23	4.16	0.25	4.74	0.39
	2kHz	4.16	0.23	4.19	0.27	5.86	0.41
	1kHz	4.82	0.23	5.74	0.31	6.24	*
	500Hz	5.25	0.74	6.12	0.98	8.41	*
V	4kHz	5.54	0.23	6.17	0.3	6.99	0.48
	2kHz	5.99	0.22	6.91\o	0.03	7.74	0.71
	1kHz	6.77	0.35	7.91	0.7	9.35	1.07
	500Hz	7.45	1.32	8.91	0.89	11.24	0.68

Note: * indicates insufficient data.

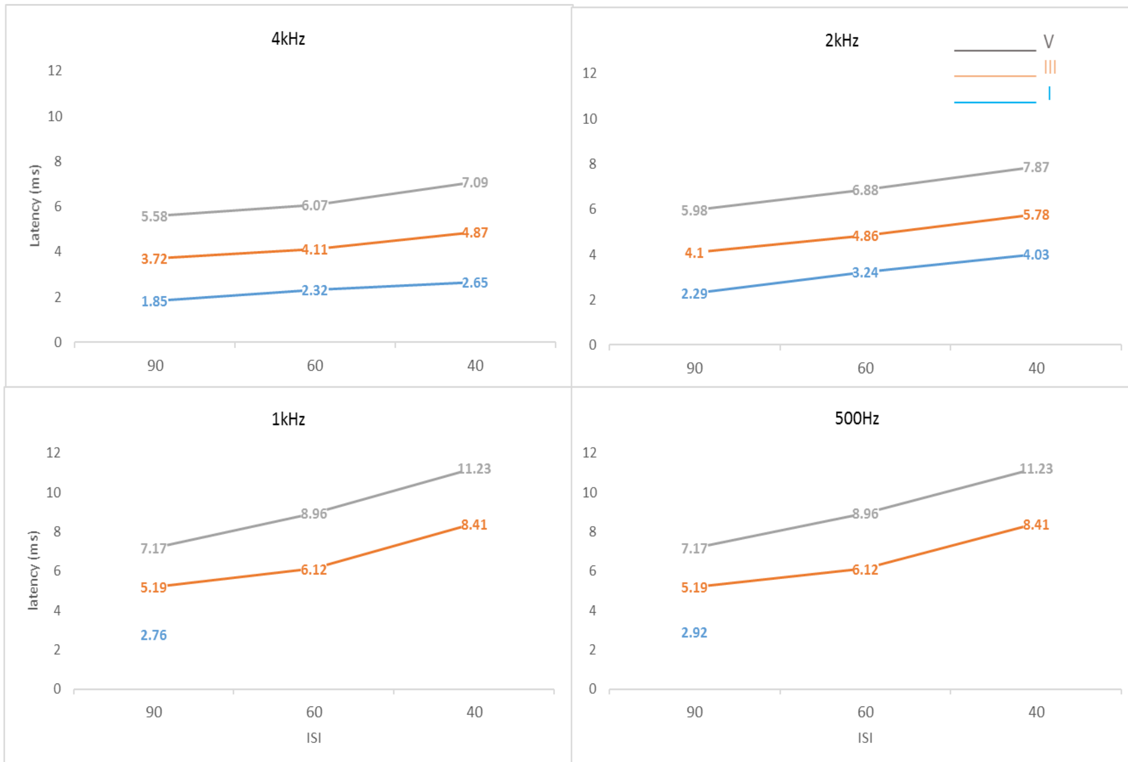

Figure 4.3: Mean latencies of waves I, III and V at 90, 60 and 40dBnHL, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz using MFABR.

Wilcoxon signed rank test was used to check whether there was any statistically significant difference across intensities. Table 4.2 shows the results for the same. Results showed a significant difference in the peak latency of all the three peaks across intensities. This was true at all the four test frequencies.

Table 4.2: Results of Wilcoxon signed rank test comparing latency of wave I, III and V across intensities, elicited with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz in MFABR

Intensity		4kHz			2kHz			1kHz		500 Hz	
		I	III	V	I	III	V	III	V	III	V
	z	2.25	3.07	3.68	1.34	3.18	3.72	2.37	3.62	1.00	3.52
90-60	p	0.012	0.023	0.001	0.18	0.001	0. 01	0.018	0.001	0.317	0.001
	z	*	2.21	3.52	*	2.524	3.517	*	3.30	*	2.33
60-40	p	*	0.027	0.001	*	0.012	0.001	*	0.001	*	0.08
	z	1.34	2.21	3.72	1.34	2.52	3.62	*	3.62	*	2.66
90-40	p	0.18	0.027	0.001	0.18	0.012	0.001	*	0.001	*	0.008

Note: * indicates insufficient data

Results of Peak Amplitude

Table 4.3 gives the median and standard deviation of peak amplitude of wave I, III and V, across three intensities, at four different frequencies, elicited using MFABR. Figure 4.4 shows the mean amplitudes of the three waves across intensities at four different frequencies, elicited using MFABR.

On comparison, it was observed that in general there was lower mean amplitude as the intensity was decreased. However, it was not a very clear and uniform trend. The results were same at all frequencies. There was no particular pattern in the way mean amplitudes varied across frequencies.

Table 4.3: Median and Standard deviation of peak amplitude (µV) of waves I, III and V at four different frequencies (4000Hz, 2000Hz, 1000Hz & 500Hz) at three intensities (90, 60 & 40 dBnHL), elicited using MFABR

Wave	Frequency of TB	90dBnHL		60dBnHL		40dBnHL	
		Median	SD	Median	SD	Median	SD
I	4kHz	0.10	0.13	0.04	0.05	0.09	0.04
	2kHz	0.05	0.08	0.05	0.02	0.03	0.07
	1kHz	0.15	0.10	0.05	*	*	*
	500Hz	0.17	0.09	*	*	*	*
III	4kHz	0.17	0.11	0.07	0.04	0.08	0.65
	2kHz	0.20	0.08	0.05	0.07	0.03	0.07
	1kHz	0.09	0.20	0.06	0.06	0.13	*
	500Hz	0.08	0.14	0.12	0.20	0.08	*
V	4kHz	0.10	0.09	0.07	0.05	0.03	0.04
	2kHz	0.08	0.07	0.08	0.08	0.00	0.08
	1kHz	0.08	0.13	0.03	0.06	0.00	0.09
	500Hz	0.18	0.19	0.02	0.12	0.08	0.05

Note: * indicates insufficient data

Wilcoxon signed rank test was used to check whether the differences in the mean amplitude observed across intensities is significantly different. Results (Table 4.4) showed significant difference across intensities.

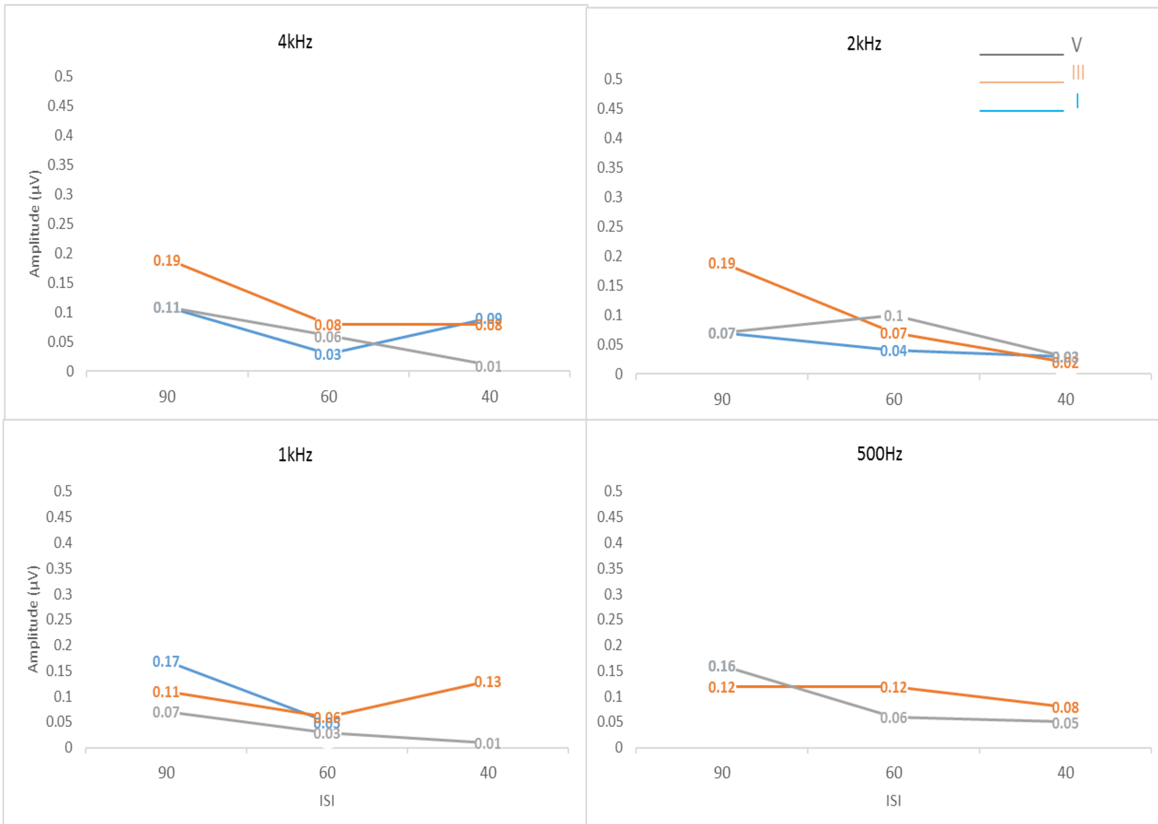

Figure 4.4: Mean amplitudes (µV) of waves I, III and V at 90, 60 and 40 dBnHL, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz using MFABR.

Table 4.4: Results of Wilcoxon signed rank test comparing amplitude of wave I, III and V across intensities, elicited with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz in MFABR

Intensity		4kHz			2kHz			1kHz		500 z	
		I	III	V	I	III	V	III	V	III	V
90-60	z	1.82	3.06	2.52	1.34	3.18	1.04	0.16	0.59	1.34	2.24
	p	0.06	0.002	0.01	0.18	0.001	0.29	0.86	0.55	0.18	0.25
60-40	z	*	0.74	2.96	*	1.33	2.94	*	1.4	*	0.53
	p	*	0.45	0.003	*	0.182	0.003	*	0.162	*	0.59
90-40	z	1.34	2.20	3.24	1.00	2.52	2.08	*	1.61	*	2.19
	p	0.18	0.02	0.001	0.31	0.12	0.37	*	0.10	*	0.008

Note: * indicates insufficient data

4.2 Comparison of MFABR across the Three Multifrequency Chains of Tone Bursts of Different ISIs

This section addresses the primary objective of the study. MFABRs were recorded across three ISIs - 20, 15 and 10ms, at 90 and 40 dBnHL. The data obtained was analysed with respect to prevalence of waves, latency and amplitude of wave I, III and V. The results are reported separately for 90 and 40 dBnHL

4.2.1 Comparison across ISIs at 90 dBnHL

Figure 4.5 shows a set of MFABRs recorded in a representative participant at 90 dBnHL across 20, 15 and 10 ms. Recordable ABRs were obtained at all the 3 ISIs in most of the participants. However, as the ISI reduced, the influence of post auricular muscle potential on the subsequent ABR of lower frequency was high. This interfered with the response analysis. Among the ones in whom ABRs could be successfully identified, wave I, III and V were analysed for their presence, latency and amplitude.

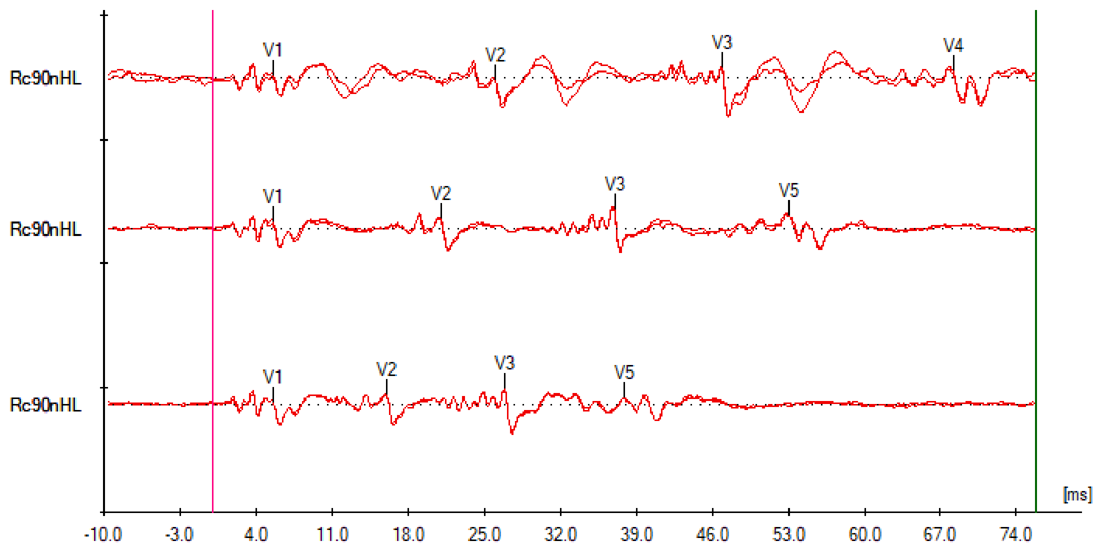

Figure 4.5: MFABRs recorded at 90dBnHL across 20ms, 15ms and 10ms ISI (in the order from top to bottom) in a representative participant.

Results of Prevalence of ABR Waves

Figure 4.6 shows the prevalence of wave I, III and V across the ISIs, at 90dBnHL, elicited by tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz in MFABR. Prevalence data shows that wave I, III and V were present in maximum number of individuals in 20 ISI. As the ISI reduced, the number of individuals with these waves reduced. Among the 3 waves, wave V was more immune to the influence of ISI compared to wave III and wave I. This was further influenced by the frequency of the tone burst. At 4000Hz, number of individuals with identifiable ABR waves was less affected by ISI. Whereas at 500Hz, as the ISI decreased, there was large decrease in the number of individuals with identifiable waves, particularly the wave I and III.

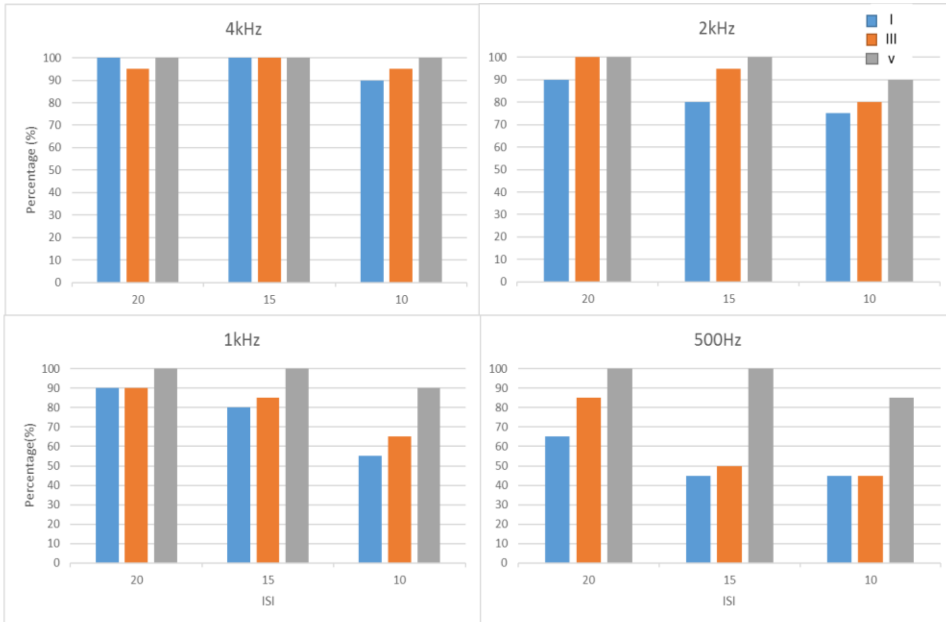

Figure 4.6: Prevalence of wave I, III and V recorded across the three ISIs at 90dBnHL, at the four test frequencies in MFABR.

Results of Peak Latency

Table 4.5 gives median and standard deviation of peak latency of wave I, III and V across the three ISIs (20, 15 & 10ms) with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz at 90 dBnHL in MFABR. Figure 4.7 shows mean latencies of wave I, III and V across ISIs in the same conditions.

Table 4.5: Median and Standard deviation of peak latency for waves I, III and V across 20, 15 and 10ms ISIs with the tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 90 dBnHL elicited using MFABR

Wave	Frequency of TB	20 ISI		15 ISI		10 ISI	
		Median	SD	Median	SD	Median	SD
I	4kHz	1.87	0.10	1.9	0.11	1.91	0.10
	2kHz	2.32	0.21	2.24	0.61	2.24	0.22
	1kHz	2.74	0.25	2.86	0.42	2.91	0.48
	500Hz	2.99	0.58	3.07	0.88	3.57	0.87
III	4kHz	3.82	0.23	3.82	0.17	3.82	0.18
	2kHz	4.16	0.23	4.24	0.30	4.33	0.27
	1kHz	4.82	0.23	4.82	0.32	4.82	0.43
	500Hz	5.25	0.74	5.20	0.70	5.41	1.09
V	4kHz	5.54	0.23	5.55	0.25	5.66	0.23
	2kHz	5.99	0.22	6.07	0.25	6.07	0.27
	1kHz	6.77	0.35	6.82	0.28	6.87	0.36
	500Hz	7.45	1.32	7.62	0.64	7.74	0.82

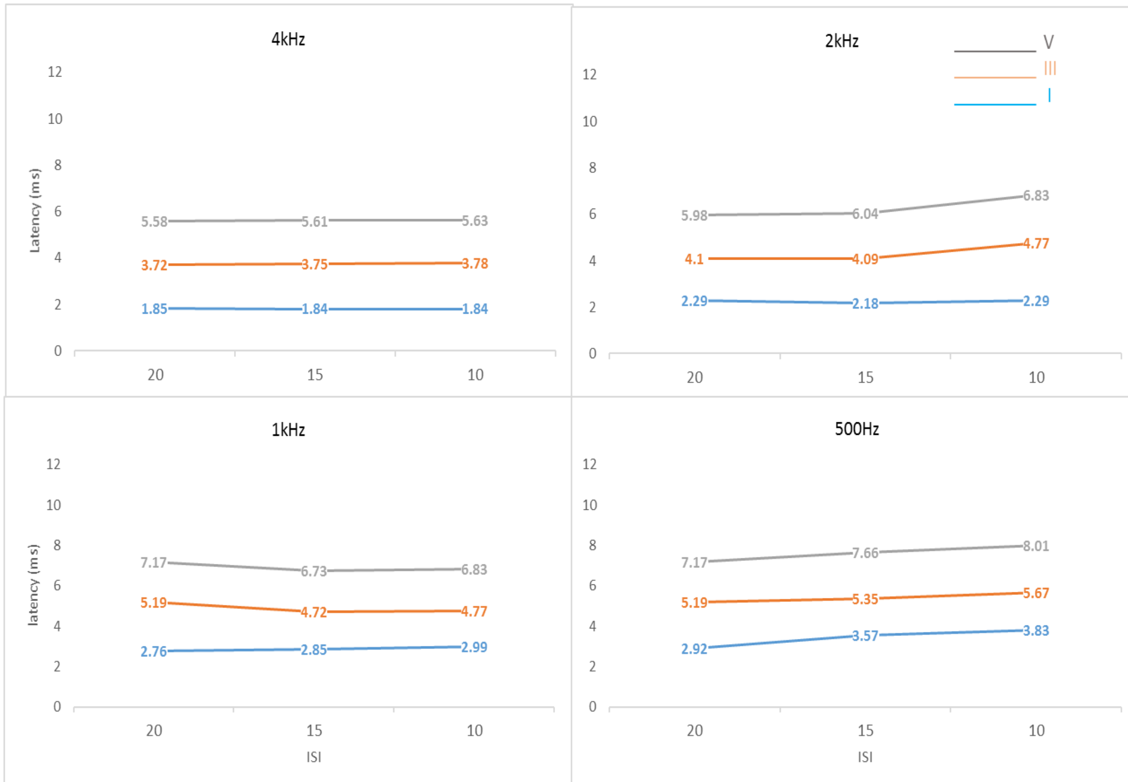

Figure 4.7: Mean latencies of wave I, III and V across 20, 15 and 10 ms ISIs, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 90dBnHL in MFABR.

In general, the data showed that as the ISI decreased, the latency was prolonged. This was true for all the frequencies and all the three waves, except for wave III and V in 1kHz. Wilcoxon signed rank test was used to check if there was a significant difference across ISIs at 90dBnHL. Table 4.6 illustrates the results obtained. The results obtained showed a significant difference in amplitude across all the three ISIs.

Table 4.6: Results of Wilcoxon signed rank test comparing peak latency for waves I, III and V across 20, 15 and 10ms, at 90 dBnHL, for tone bursts of 4kHz, 2kHz, 1kHz and 500 Hz in MFABR

ISI		4kHz			2kHz			1kHz			500 Hz		
		I	III	V	I	III	V	I	III	V	I	III	V
20-15	z	0.08	7.10	1.26	0.039	0.07	1.82	0.71	0.10	0.62	0.42	0.51	1.76
	p	0.93	0.47	0.20	0.96	0.93	0.06	0.47	0.91	0.53	0.67	0.61	0.07
15-10	z	0.18	1.46	1.13	0.61	2.62	0.74	0.21	0.34	0.25	0.00	0.67	2.09
	p	0.85	0.14	0.25	0.54	0.009	0.45	0.83	0.73	0.01	1.00	0.49	0.36
20-10	z	0.53	0.15	2.31	1.02	2.49	1.39	0.95	0.42	1.65	0.94	1.40	2.64
	p	0.59	0.87	0.02	0.30	0.13	0.16	0.34	0.67	0.09	0.34	0.16	0.008

Results of Peak Amplitude

Table 4.7 gives median and standard deviation of peak amplitude of wave I, III and V across the three ISIs (20, 15 & 10ms) with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz at 90 dBnHL in MFABR. Figure 4.8 shows mean peak amplitude of wave I, III and V across ISIs in the same conditions.

The mean and median data did not show any specific trend in the way peak amplitudes across the three ISIs at 90dBnHL. In several instances amplitude of the waves was better in 15 ISI compared to 20 and 10 ISI. Results of Wilcoxon (Table 4.8) showed significant difference in the amplitude of the waves across all the three ISIs.

Table 4.7: Median and Standard deviation of peak amplitude (µV) for waves I, III and V across 20, 15 and 10ms ISIs with the tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 90 dBnHL elicited using MFABR

Wave	Frequency of TB	20 ISI		15 ISI		10 ISI	
		Median	SD	Median	SD	Median	SD
I	4kHz	0.10	0.13	0.10	0.11	0.14	0.11
	2kHz	0.05	0.08	0.17	0.17	0.12	0.21
	1kHz	0.15	0.10	0.10	0.24	0.10	0.19
	500Hz	0.17	0.09	0.17	0.15	0.08	0.15
III	4kHz	0.17	0.11	0.20	0.21	0.14	0.13
	2kHz	0.20	0.08	0.17	0.16	0.14	0.28
	1kHz	0.09	0.20	0.17	0.28	0.14	0.16
	500Hz	0.08	0.14	0.20	0.19	0.15	0.20
V	4kHz	0.10	0.09	0.07	0.10	0.07	0.82
	2kHz	0.08	0.07	0.15	0.12	0.16	0.14
	1kHz	0.08	0.13	0.16	0.15	0.15	0.23
	500Hz	0.18	0.19	0.25	0.21	0.12	0.23

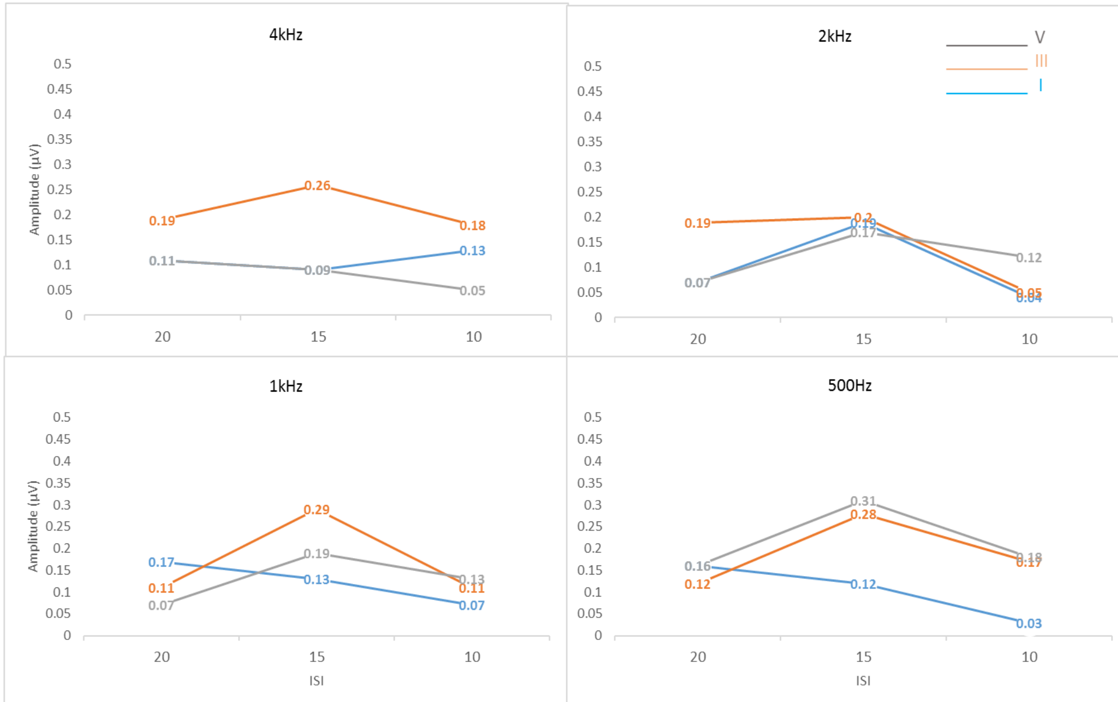

Figure 4.8: Mean peak amplitude (µV) for wave I, III and V recorded at 90 dBnHL across 20, 15 and 10 ms ISI, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz using MFABR.

Table 4.8: Results of Wilcoxon signed rank test comparing peak amplitudes for waves I, III and V (4000Hz, 2000Hz, 1000Hz & 500Hz) at 90 dBnHL across 20, 15 and 10ms elicited using MFABR

ISI		4kHz			2kHz			1kHz			500 Hz		
		I	III	V	I	III	V	I	III	V	I	III	V
20-15	z	0.54	1.02	0.28	2.02	0.02	2.43	0.50	2.01	2.4	0.56	2.25	2.44
	p	0.58	0.30	0.77	0.04	0.09	0.01	0.61	0.04	0.01	0.57	0.02	0.01
15-10	z	0.61	0.84	1.04	1.78	1.61	0.36	0.77	1.73	1.19	0.67	2.02	2.88
	p	0.54	0.4	0.29	0.07	0.1	0.71	0.44	0.08	0.23	0.5	0.04	0.004
20-10	z	0.82	0.42	2.17	0.35	2.38	1.13	1.32	0.03	1.49	1.77	1.18	0.45
	p	0.4	0.6	0.03	0.72	0.01	0.25	0.18	0.96	0.13	0.07	0.23	0.65

4.2.2 Comparison across ISIs at 40 dBnHL

Figure 4.9 shows a set of MFABRs recorded in a representative participant at 40 dBnHL across 20, 15 and 10 ms of ISI. Recordable ABRs were obtained at all the 3 ISIs in most of the participants. The results are reported separately for prevalence of waves, their peak latency and peak amplitudes.

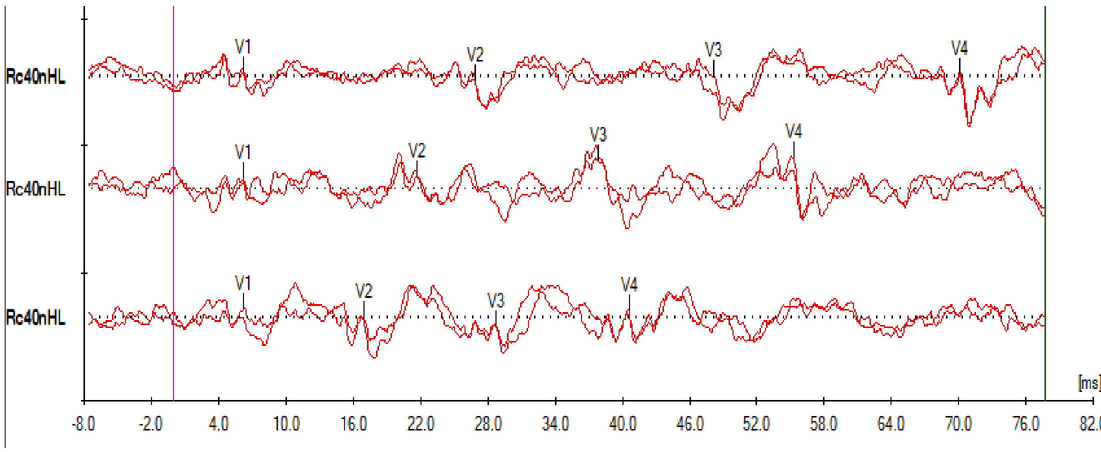

Figure 4.9: MFABRs recorded at 40dBnHL across 20ms, 15ms and 10ms ISI (in the order from top to bottom) in a representative participant.

Results of Prevalence of ABR Waves

Figure 4.10 shows the prevalence of wave I, III and V across the ISIs, at 40dBnHL, elicited by tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz in MFABR. Prevalence data shows that wave I, III and V were present in maximum number of individuals in 20 ISI. As the ISI reduced, the number of individuals with these waves reduced. Among the 3 waves, wave V was more immune to the influence of ISI compared to wave III and wave I. This was further influenced by the frequency of the tone burst. At 4000Hz, number of individuals with identifiable ABR waves was less affected by ISI. Whereas at lower frequencies, as the ISI decreased, there was large decrease in the number of individuals with identifiable waves, particularly the wave I and III.

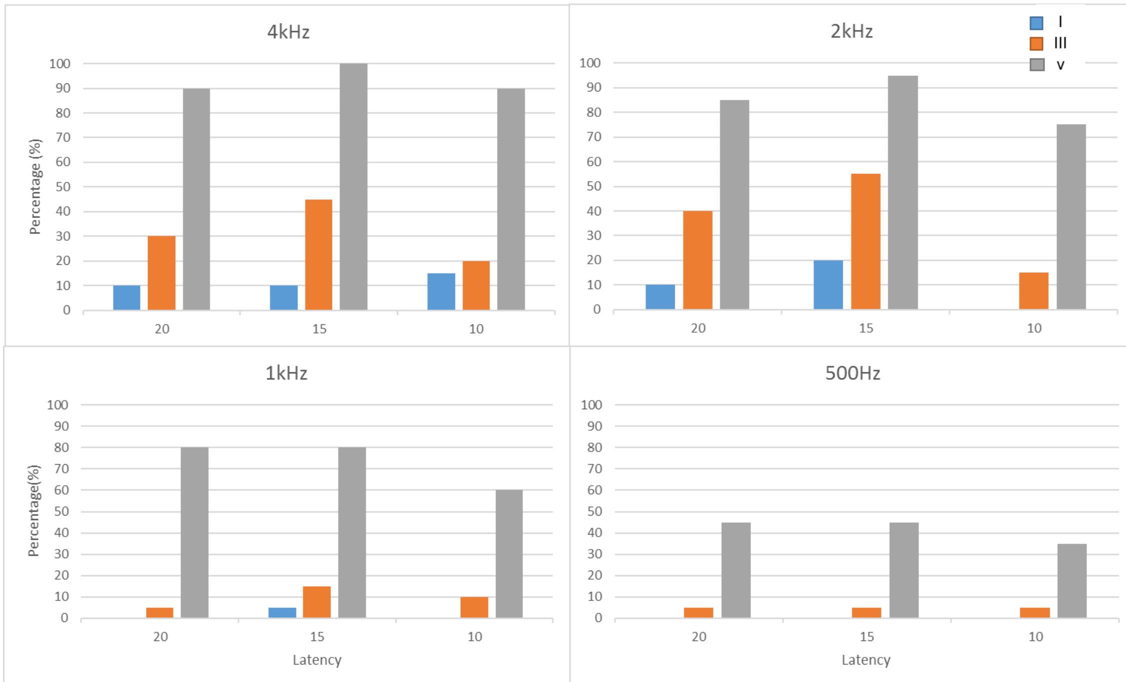

Figure 4.10: Prevalence of wave I, III and V recorded across the three ISIs at 40dBnHL, at the four test frequencies in MFABR.

Results of Peak Latency

Table 4.9 gives median and standard deviation of peak latency of wave I, III and V across the three ISIs (20, 15 & 10ms) with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz at 40 dBnHL in MFABR. Figure 4.11 shows mean latencies of wave I, III and V across ISIs in the same conditions.

Among the less number of participants who showed ABR waves present, the mean or median data did not show any consistent trend in the way peak latency varied across ISIs. Results of the Wilcoxon sign rank test (Table 4.10) did not show any significant difference in the median peak latency across the three ISIs. This was true for all the frequencies of tone bursts.

Table 4.9: Median and Standard deviation of peak latency for waves I, III and V across 20, 15 and 10ms ISIs with the tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 40 dBnHL elicited using MFABR

Wave	Frequency of TB	20 ISI		15 ISI		10 ISI	
		Median	SD	Median	SD	Median	SD
I	4kHz	2.65	0.23	2.99	0.11	2.99	0.35
	2kHz	4.03	0.53	3.82	0.55	*	*
	1kHz	*	*	5.74	*	*	*
	500Hz	*	*	*	*	*	*
III	4kHz	4.74	0.39	4.91	0.55	4.49	0.38
	2kHz	5.86	0.41	5.74	0.47	5.16	0.55
	1kHz	6.24	*	6.07	0.72	6.49	0.46
	500Hz	8.41	*	8.49	*	8.66	*
V	4kHz	6.99	0.48	6.91	0.48	6.99	0.39
	2kHz	7.74	0.71	7.74	0.61	7.82	0.67
	1kHz	9.35	1.07	9.32	0.95	9.69	1.18
	500Hz	11.24	0.68	11.91	1.44	9.77	1.03

Note: * indicates insufficient data

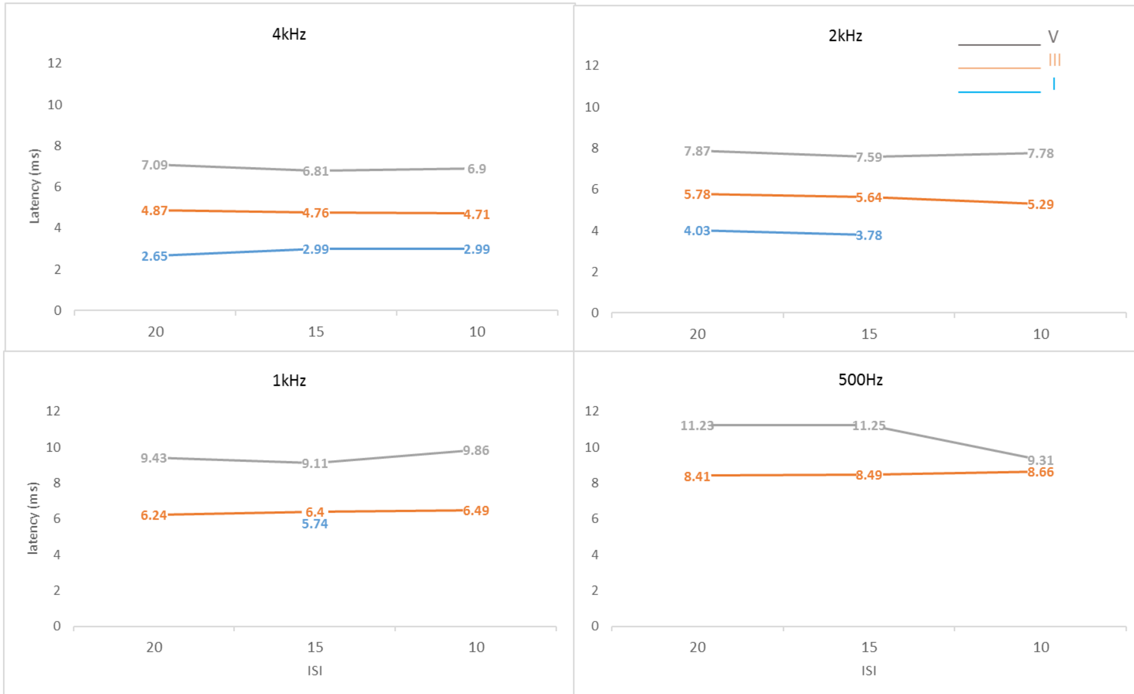

Figure 4.11: Mean peak latencies of wave I, III and V across 20, 15 and 10 ms ISIs, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 40dBnHL in MFABR.

Table 4.10: Results of Wilcoxon signed rank test comparing peak latency for waves I, III and V across 20, 15 and 10ms, at 40 dBnHL, for tone bursts of 4kHz, 2kHz, 1kHz and 500 Hz in MFABR

ISI		4kHz			2kHz			1kHz			500 Hz		
		I	III	V	I	III	V	I	III	V	I	III	V
20-15	z	*	0.18	1.79	0.44	0.73	0.82	*	*	1.49	*	*	0.81
	p	*	0.85	0.73	0.65	0.46	0.4	*	*	0.13	*	*	0.41
15-10	z	*	*	0.62	*	0.0	1.57	*	*	1.68	*	*	1.48
	p	*	*	0.53	*	1.0	0.11	*	*	0.09	*	*	0.13
20-10	z	*	1.00	1.00	*	0.44	0.11	*	*	0.94	*	*	1.06
	p	*	0.31	0.31	*	0.65	0.90	*	*	0.34	*	*	0.28

Note: * indicates insufficient data

Results of Peak Amplitude

Table 4.11 gives median and standard deviation of peak amplitude of wave I, III and V across the three ISIs (20, 15 & 10ms) with tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz at 40 dBnHL in MFABR. Figure 4.12 shows mean peak latencies of wave I, III and V across ISIs in the same conditions.

Table 4.11: Median and Standard deviation of peak amplitude (µV) for waves I, III and V across 20, 15 and 10ms ISIs with the tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 40 dBnHL elicited using MFABR

Wave	Frequency of TB	20 ISI		15 ISI		10 ISI	
		Median	SD	Median	SD	Median	SD
I	4kHz	0.09	0.04	0.01	0.04	0.07	0.03
	2kHz	0.03	0.07	0.10	0.11	*	*
	1kHz	*	*	0.07	*	*	*
	500Hz	*	*	*	*	*	*
III	4kHz	0.08	0.65	0.02	0.06	0.07	0.06
	2kHz	0.03	0.07	0.14	0.05	0	0.02
	1kHz	0.13	*	0.13	0.06	0.03	0.07
	500Hz	0.08	*	0.18	0.00	0.02	*
V	4kHz	0.03	0.04	0.02	0.06	0.05	0.05
	2kHz	0.00	0.08	0.07	0.04	0.05	0.07
	1kHz	0.00	0.09	0.07	0.09	0.04	0.07
	500Hz	0.08	0.05	0.18	0.09	0.02	0.09

Note: * indicates insufficient data

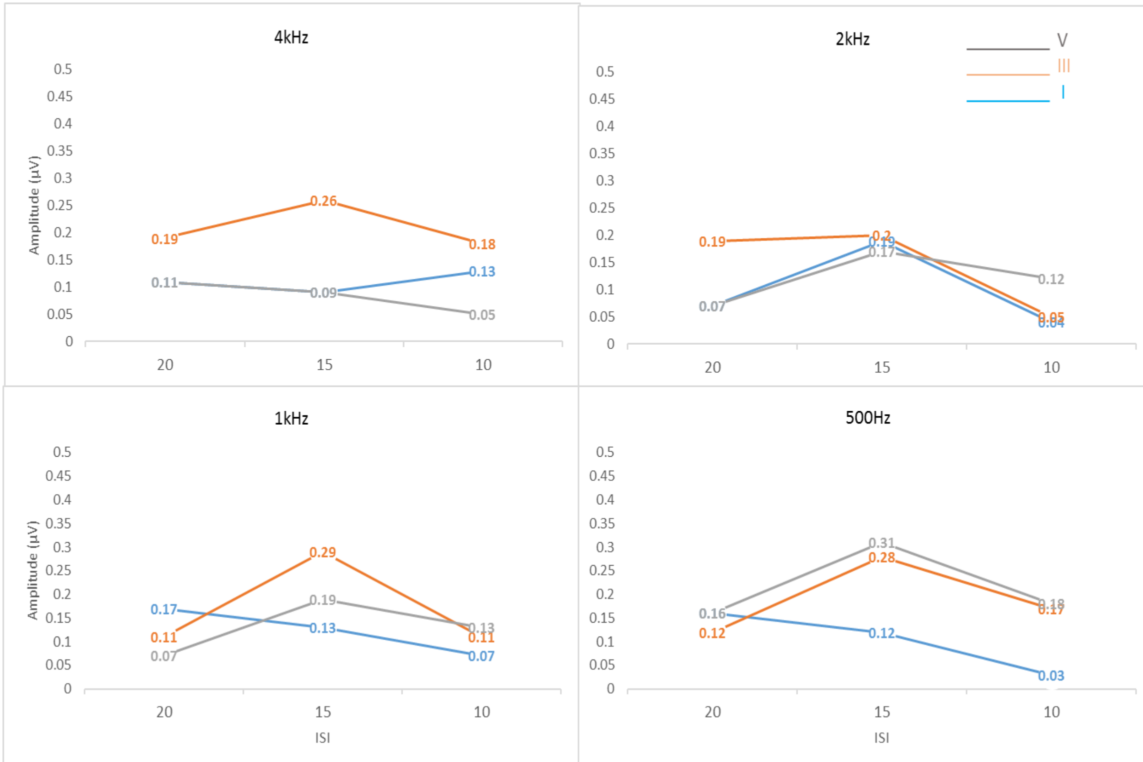

Figure 4.12: Mean amplitudes (µV) of wave I, III and V across 20, 15 and 10 ms ISIs, recorded for tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz, at 40dBnHL in MFABR.

The mean and median data did not show any specific trend in the way peak amplitudes across the three ISIs at 40dBnHL. In several instances amplitude of the waves was better in 15 ISI compared to 20 and 10 ISI.

To check if there was any statistical difference in the peak amplitude across ISIs at 40dBnHL, Wilcoxon signed rank test was used. The results obtained are shown in Table 4.12. Results showed a significant difference between 20 and 15 ISIs for wave V at 1kHz and 500Hz tone bursts.

Table 4.12: Results of Wilcoxon signed rank test comparing peak amplitude for waves I, III and V across 20, 15 and 10ms, at 40 dBnHL, for tone bursts of 4kHz, 2kHz, 1kHz and 500 Hz in MFABR

ISI		4kHz		2kHz		1kHz			500 Hz	
		III	V	I	III	V	III	V	III	V
20-15	z	1.60	1.09	*	1.99	3.31	*	1.53	*	2.02
	p	0.1	0.27	*	0.46	0.001	*	0.12	*	0.04
15-10	z	*	0.94	*	1.60	1.29	1.34	1.22	*	1.76
	p	*	0.34	*	0.10	0.19	0.18	0.22	*	0.07
20-10	z	0.0	1.14	*	1.0	1.50	*	0.35	*	0.53
	p	1.0	0.25	*	0.31	0.13	*	0.72	*	0.59

Note: * indicates insufficient data

Chapter 5

DISCUSSION

The MFABR has been shown to be a useful method for objective estimation of frequency-specific hearing thresholds. The MFABRs allow to estimate frequency-specific hearing thresholds at all frequencies almost simultaneously, using a chaining approach. Here, tone-bursts of different frequencies are chained together with a small temporal gap. The chained stimuli are then presented repetitively in order to obtain averaged ABR responses. The small temporal separation of two successive tone-bursts of different frequencies ensures that the stimuli and their corresponding ABR do not overlap in time.

In conventional ABR recordings, we present stimuli with a repetition rate of approximately 9.1 /s or 11.1/s. Such a repetition rate would lead to an ISI of ~100 ms. This ISI is used for every frequency which linearly increases the time taken for completing the ABR recording. In the MFABR however, the idea that of frequency-specific ISI is utilized. Here the ISI per frequency is maintained the same as conventional ABR recordings, however the ISI between tone-bursts of two different frequencies is reduced. This is based on the principle of frequency tuning. The inner hair cells and auditory neurons respond maximally to a stimulus matching their characteristic frequency. This occurs because of the tuning of the basilar membrane and its travelling wave. Due to such a phenomenon, we can stimulate the inner hair cell and auditory neurons of certain frequency region without stimulating the rest of the regions.

In conventional ABR recordings, a stimulus is presented only after allowing sufficient refractory period after the preceding stimulus. In the MFABR technique, during the refractory period for one frequency region, a tone-burst of another frequency is presented. This ensures that the time that we normally lose in waiting for the neurons to recover is essentially utilized by testing a different set of neurons. Thus, the technique is time-efficient and aides in the recording of frequency-specific ABRs.

The MFABR technique is novel technique and there have been only a handful of studies that have evaluated its utility in hearing assessment (Mamatha, 2016; Swathy, 2017). Before a technique is implemented in the clinical scenario, it is imperative to thoroughly evaluate the different parameters involved. Previous studies have used an ISI of 20 ms between successive ton-bursts in a chain. However, the effect of varying this ISI was not evaluated. In the current study, the effect of varying the ISI between successive tone bursts in a stimulus chain in MFABR was evaluated. The effect of varying the ISI was evaluated at a supra threshold intensity of 90 dB nHL, and also at 40 dB nHL. The 40 dB nHL was considered as it is the lower intensity at which ABR is performed for hearing screening. The ISIs used were 20ms, 15ms and 10 ms. Additionally the MFABR with ISI of 20 ms was recorded at 60 dB nHL also. This served as a direct replication of Mamatha (2016) study, and aided in validating the MFABR technique. The findings of the present study are reported under the following headlines

1. Comparison of MFABR across intensities at ISI of 20ms
2. Comparison of MFABR across the three multifrequency chains of tone bursts of different ISIs

5.1 Comparison of MFABR Across Intensities at ISI of 20 ms

An increase in the latency of the waves was seen as the frequency of the tone-burst was decreased. Similar findings have been reported for frequency-specific ABRs using tone bursts (Gorga, Kaminski & Jesteadt, 1988; Fausti, Mitchell, Frey, Henry & O'Connor, 1994). Increase in latency with decreasing tone-burst frequency represents the time taken for the cochlear travelling wave to travel from 4000Hz to 500Hz (Gorga et al., 2006). For wave V, the average prolongation seen at 4kHz, 2kHz, 1kHz and 500 Hz was 1.51 ms, 1.89 ms, 2.67 ms and 4.06 ms respectively.

Results revealed a decrease in the mean amplitudes of waves I, III and V as the intensity reduced. This was seen at all the frequencies tested. The variation in amplitude ranged from 0.02 µV to 0.17 µV. Larger variation in amplitude across the frequencies were seen at 40 dB nHL, when compared to 60 and 90 dB nHL. Such a trend has been reported earlier in tone-burst evoked ABRs using conventional methods. The reduction in amplitudes across with decrease in intensity attributed to decreased excitation pattern, which leads to reduction in the number of neurons that respond to the stimulus. This reduction in the number of neurons lead to a decrease in the amplitude of the compound action potential.

The pattern of results of MFABR across intensities follows the same trend as in the study by Mamatha (2016). This suggests the results of MFABR are replicable across studies. Such replicability across studies strengthens the validity of the MFABR technique in hearing threshold estimation.

5.2 Comparison of MFABR Across ISIs

MFABR latencies and amplitudes were compared across ISIs. Decreasing ISIs led to prolongation in latency and decrease in amplitude of the ABR waves. The prevalence of waves I, III and V reduced as a function of the ISI. Additionally, the morphology of the MFABR waves were poorer in recordings with shorter ISIs. The poorer morphology in the short ISI recordings hindered the identification of the ABR waves, which was especially seen at lower intensities.

When the interstimulus interval is reduced, the inner hair cell and auditory neurons do not get sufficient time to replenish their supply of neurotransmitters. Due to this there is a delay in the neural response to the next stimulus. This delay is also associated with a decrease in amplitude of the ABR waves.

In the MFABR stimulus, the ISI between successive frequencies within the stimulus chain was varied. This stimulus chain was presented repetitively to get averaged ABR responses. The repetition rate for all the was kept constant at 9.1 /s, irrespective of the change in ISI within the stimulus chain. Due to such a manipulation the frequency-specific ISI across trials did not change, even when the ISI within the chain was varied. This ensured that the inner hair cell and the auditory neurons responding do not experience differing amounts of fatigue due to change in the ISI within the stimulus chain. However, due to the reduction in the ISI within the stimulus chain, the preceding stimulus might influence the response to the successive stimulus within the chain. This would occur because of the spread of excitation due to the tone-burst. When there is an excitation spread by the preceding tone-burst, the neurons responding to successive tone-burst are also excited. In the current study 4000 Hz preceded 2000 Hz in a chain. Excitation spread from 4000 Hz might lead to off-frequency excitation of the 2000 Hz region. After the 4000 Hz stimulus, when the 2000 Hz stimulus is presented in the chain, the neurons in the 2000 Hz region that were had already fired due to off-frequency excitation, have to fire again for the on-frequency stimulus. Such a phenomenon would also occur for the 1000 Hz and 500 Hz stimulus also. However, the same would not occur for the 4000 Hz (the first stimulus in the chain), as the first stimulus in a chain is always preceded by a silent interval of ~>41 ms (the lowest inter-stimulus interval between two successive stimulus chains). Based on this, it was expected that the change in inter-stimulus interval in the MFABR recording would not affect the responses to the first stimulus in the chain, which was 4000 Hz in the current study. Axiomatically, it was expected that the change in ISI would affect the responses to the later stimuli in the chain, which were 2000 Hz, 1000 Hz, and 500 Hz in the current study.

Contrary to the expectation detailed above, there was no clear trend in the effect of ISI on the latencies and amplitude of the MFABRs. The latencies of wave V were generally longer for ISIs of 10 ms across frequencies. This prolongation in latency did not exceed a median difference of 0.3 ms. This suggests that an ISI of 10 ms in MFABR is not preferable for use in clinical applications that require precise latency measurements as the diagnostic criteria, such as site-of-lesion testing using stacked ABR etc. In addition to the increase in latency, peak amplitudes were low at ISIs of 10 ms compared to 20 ms and 15 ms. This trend again, was not clearly distributed across frequencies. Similar to the latency measures, the amplitude measures of MFABR recorded using ISIs of 10 ms cannot serve as reliable clinical tools when precise amplitude measurements are necessary to make clinical judgments. Thus, the MFABR can recorded with ISIs of 15 ms and 20 ms in clinical practice for diagnostic evaluation of hearing sensitivity as well as for determining the integrity of the auditory neural pathway.

Another parameter, i.e., the prevalence of waves in the MFABR across ISIs was also evaluated. This is a particularly important parameter for hearing threshold estimation. Prevalence of MFABR were similar for ISIs of 20 ms and 15 ms. At ISI of 10 ms however, the prevalence of the MFABR waves was very low. Earlier it was mentioned that the median prolongation of wave V latency at ISI of 10 ms did not exceed ~0.3 ms. Here, the actual prolongation might have been under-estimated because of the missing latency values due to the absence of ABR waves in the 10 ms ISI recordings.

The absence of ABR waves in the 10 ms ISI recordings could be attributed to the refractory period of the auditory nerves and the inner hair cell. Taxing the refractory period of the auditory nerves would have led to reduction in the amplitude of ABR waves. Reduction in amplitude of the ABR waves lead to poorer signal to noise ratios. The poor SNR is visualized in the ABR, as waveforms with poor morphology. Identification of ABR waves is difficult in recordings with poor morphology. Thus, reduction in wave amplitude due to short ISI might be one of the reasons for lower prevalence of waves I, II and V in recordings with ISI of 10 ms.

It was also noted that recordings with ISI of 10 ms were more susceptible to contamination by the postauricular muscle response (PAM). This was seen, as the latencies of the ABRs coincided with latencies of the PAM to the previous stimulus in the chain, due to short ISI. On the other hand, presence of PAM in the recordings with 15 and

20 ms ISI did not obscure the MFABRs. Figure 5.1 shows an MFABR waveform from a representative participant where the PAM obscured the identification of the ABR waves.

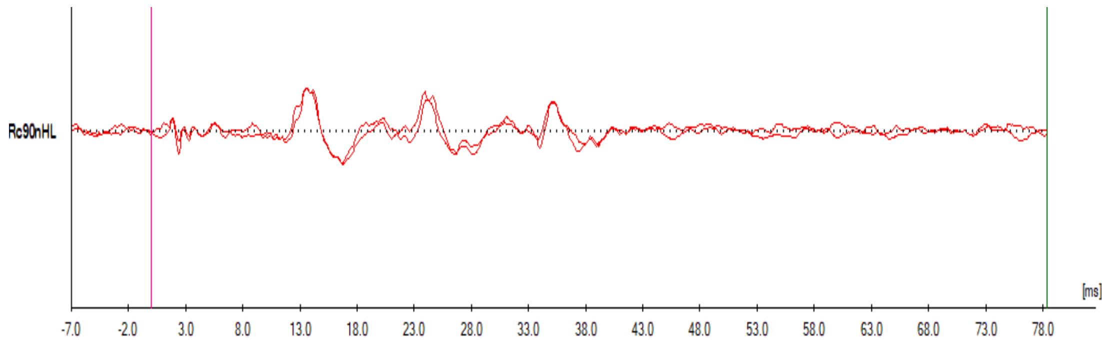

Figure 5.1: Presence of PAM in MFABR recorded with 10ms ISI in a representative participant.

In hearing threshold estimation, it is the presence or absence of the ABR waves at different intensities, which is used for making clinical judgments. As the prevalence of MFABR waves was very low in MFABRs recorded using ISIs of 10 ms, it is not advisable to use this ISI in clinical MFABR recordings. Based on the findings of the current study, the use of ISIs of greater than 15 ms is suggested for clinical use of MFABRs for hearing threshold estimation.

Chapter 6

SUMMARY AND CONCLUSIONS

The utility of MFABR as a time efficient clinical tool to record frequency specific information using an onset to onset ISI of 20ms has already been established (Mamatha, 2016; Swathy, 2017). However, using an onset to onset ISI of 20ms enable us to record MFABRs at not higher 10/sec stimulus rate. If the ISI could be reduced without affecting the responses, it would help us record MFABRs at rates higher than 10/sec. Hence, the present study was taken up to probe into the possibility of further improving the time efficiency of MFABR.

MFABRs were recorded in 20 normal hearing adults using the protocol recommended by Mamatha (2016). Three chain of tone bursts differing in the onset to onset interval (ISI) were generated and used to elicit MFABRs. The three ISIs used were 20ms, 15ms and 10ms. The MFABRs were recorded for all the three stimulus chains with stimulus intensity being 90 and 40dBnHL. For the stimulus chain with 20 ISI, an additional recording at 60 dBnHL was carried out. The waveforms obtained were analysed for the presence of wave I, III and V. If the waves were present, their peak latency and amplitude were noted down. This was done for ABRs elicited by tone bursts of 4000Hz, 2000Hz, 1000Hz and 500Hz.

MFABRs could be successfully recorded at all the three ISIs in all the participants. However, number of participants with ABR waves present decreased as the ISI reduced. This was seen more so at lower frequencies and in wave I and III. Wave V on the other hand was less immune to the effects of reduced ISI. Such effects of ISI was more evident at 40dBnHL compare to 90dBnHL.

IBM SPSS version 23 was used to analyse the latency and amplitude data. Shapiro-Wilk test of normality revealed that the data was not normally distributed. Therefore, Wilcoxon signed rank test was used for within group comparisons. Results showed that there was no uniform trend in the way latency and amplitude varied across the ISIs. There were very few parameters that showed significant differences in peak latency and peak amplitude. But such differences were sporadically distributed.

The findings show that presence of ABR waves is affected when ISI is reduced. Such influence has immense implication for its utility in hearing threshold estimation. The absence of ABR waves at lower ISI suggests that the threshold of ABR is elevated. Therefore, MFABR elicited with lower ISI is likely to overestimate the hearing loss. Furthermore, while recording MFABRs with 10 ISI, there were significant interferences from post auricular muscle artifacts on the subsequent tone burst ABRs. This would spare the 4kHz ABR but interrupt with the ABRs for frequencies lower than that.

Based on these findings, it is advisable to use longer ISI of 15 ms and above in MFABR, in view of good recordings of ABRs and accurate hearing threshold estimation. Future studies can focus on developing the algorithms to delink the ABRs generated for different tone bursts in order to reduce the interference of post auricular muscle artefact.

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