EFFECT OF DIFFERENT ARTIFACT REJECTION LEVEL ON ABRs AND ALLRs

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CERTIFICATE

This is to certify that this dissertation entitled **'Effect of different artifact rejection levels on ABRs and ALLRs'** is a bonafide work submitted in part-fulfillment for degree of Master of Science (Audiology) of the student Registration Number: 17AUD030. 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.

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CERTIFICATE

This is to certify that this dissertation entitled **'Effect of different artifact rejection levels on ABRs and ALLRs'** has been prepared under my supervision and guidance. It is also 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 **'Effect of different artifact rejection levels on ABRs and ALLRs'** is the result of my own study under the guidance of Dr. Sandeep M., Associate Professor in Audiology, department of Audiology, 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.

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CHAPTER-1

INTRODUCTION

Hearing threshold estimation plays an important role in appropriate diagnosis of the audiological conditions and aural rehabilitation. Auditory evoked potentials (AEPs) are objective methods that help in estimating reliable hearing thresholds and also in identifying diffuse or space occupying lesions of the auditory neural pathway (Don, Masuda, Nelson & Brackmann, 1997; Chandrasekhar, Brackmann & Devgan, 1995). In research, AEPs are used to study the neural mechanisms of peripheral and central auditory processing under various stimulus conditions or listening conditions. It is well known that the recordings of AEPs will be affected by various stimulus parameters and acquisition parameters (Hall, 1992).

AEPs are always recorded in the presence of unwanted background EEG activities that are not a part of the response (Picton, Woods, Braun & Healey, 1976). These background activities should be eliminated and their influence on the AEPs needs to be minimized during the acquisition of AEPs. The background activity, also commonly known as artifacts, can be electromagnetic in nature, a stimulus artifact, electrical line artifact or muscular in its origin (Hall, 2007). The most frequently encountered among these are the muscle related artifacts.

Muscle artifacts can be a part of ongoing background activity during recordings or it can be evoked by the stimulus itself (for example post auricular muscle reflex). If muscle activity generates potentials that have the same frequency as that of the target AEPs, it is likely to be picked up, amplified, and averaged (Hall, 2007; Picton et al.,

1976), which as a result negatively affects the signal-to-noise ratio (SNR) of the recorded waveform. These potentials are relatively large in amplitude and can either be from single or multiple muscle sites in the body. Some muscle activities that are known to affect AEP recordings include eye blinks, teeth clenching, neck stiffening, limb movement, swallowing, and so on (Maruthy, Gnanateja, Ramachandran and Thuvassery (2015); Jacobson, 1994; Hall, 2007; Sokolov, Kurtz, Steinma, Long & Sokolova, 2005).

SNR of an AEP recording refers to the ratio between the magnitude of evoked potential and that of the background EEG activity. SNR can be calculated by the root mean square amplitudes of the signal, using peak to peak amplitude of the response waveform (Burkard et al., 2007) or by using a split sweep method that estimates the SNR based on the cross-correlation between two averaging buffers (Bershad & Rockmore, 1974). Good SNR is very important for the accurate detection and marking of the AEPs which in turn influences the inferences drawn from it. Therefore, during AEP recordings every attempt is made to minimize the influences of background activity and to enhance the response and SNR. This is achieved by adopting several signal enhancement strategies such as averaging, filtering and artifact rejection (Kavanagh & Franks, 1989; Hall, 2007; Hood, 1998; Jacobson, 1994). Averaging is one of the most commonly used techniques to improve SNRs in AEP recordings. Signal averaging theoretically reduces noise by the square root of the number of sweeps in the averaged response (Don, Elberling & Waring, 1984; Sanchez & Gans, 2006). Similarly, filtering provides a minimal improvement of the SNR because the frequency spectrum of noise often overlaps with the frequency composition of the AEPs (Boston & Ainslie, 1980; Elton, Scherg & Von Cramon, 1984).

Artifact rejection is a technique which excludes the unwanted responses from the ongoing average (Hood, 1998). Artifact rejection level (ARL) is set so that the unwanted potentials whose amplitude is exceeding predetermined amplitude are rejected and are not included in the averaging process of response waveform of AEPs (Hood, 1998; Hall, 2007; Stecker, 2002). It is important to note that the ARL set is usually different for different AEPs. Generally, the ARL for auditory brainstem response (ABR) is set around ±25 µV and for cortical potentials such as auditory late latency response (ALLR), it is kept around ±50 µV (Hall, 2007). Setting up an optimum ARL for the recording of different auditory evoked potentials is very essential for obtaining a waveform with good SNR.

1.1 Justification for the Study

It is known that the averaged AEP waveforms are affected by the muscular artifacts and the SNR is poor in the presence of muscle artifacts. Setting optimum ARL is meant to help in eliminating the unwanted responses from the averaged response waveform. Maruthy, Gnanateja, Ramachandran and Thuvassery (2015) reported that the ARL can be kept at ±20 µV to obtain best AEPs with good SNR. In each recording, they monitored the online EEG to set the minimum artifact rejection level such that an average response of 1000 sweeps in case of ABR and 250 sweeps in the case of LLR could be recorded with less than 10% sweep rejections. Based on their findings, they inferred that lower ARL will result in averaged waveforms of better SNRs. However this was not supported with direct evidence, as they did not record averaged waveforms. This warranted a study wherein the effect of ARL on the characteristics of averaged waveforms is systematically investigated.

Don and Elberling (1994) reported that strict ARL will affect ABR test efficiency. They reduced the ARL from ±10 µV to ±2.5 µV in different step sizes. They found that reducing the ARL reduced the background noise significantly but the overall quality of the response waveform was affected and the SNR did not improve with the reduction in ARL. Therefore, appropriate ARL need to be set in order to preserve the response and to remove the background activity. If the ARL is set higher than required, background activity is likely to get added to the averaged response and the SNR is likely to reduce. On the contrary, if the ARL is set lower than required, the responses with high amplitudes will get eliminated from the recording, leading to poor quality of response waveforms (Lightfoot & Stevens, 2013 & Maruthy et al., 2015). Therefore it is important to systematically study the effect of ARL on the characteristics of AEPs and in the process, identify the most appropriate ARL for AEP recordings.

Therefore, the present study is taken up to study the effect of different ARL on the characteristics of AEPs. Considering the extensive clinical utility of ABR and ALLR the effect of ARL was studied on these two AEPs. Typically these responses are analyzed in terms of latency, amplitude and replicability. Therefore these response parameters were of interest while studying the effect of ARL on AEPs.

1.2 Aim of the Study

The aim of the present study was to identify the optimal ARL to record Auditory Brainstem Responses and Auditory Long Latency Responses.

1.3 Objectives of the Study

1. To investigate the effect of different ARL on the averaged ABRs in terms of its latency, amplitude, replicability and SNR.

2. To investigate the effect of different ARL on the averaged ALLRs in terms of its latency, amplitude, replicability and SNR.

CHAPTER-2

REVIEW OF LITERATURE

The utility of Auditory evoked potentials (AEPs) for clinical as well as research purposes is highly appreciated. However, it is important to obtain AEPs with least interference from the other background EEG activities, which is a challenge in many instances. Optimizing the stimulus and recording parameters is the key to it and the chapter provides brief review about the different strategies to enhance signal to noise ratio (SNR) of recording. The specific focus is on the Artifact rejection level (ARL) and its influence on the AEP recordings. The information is provided under following broad headings

1. The need for enhancing SNR of AEP recordings
2. Techniques to enhance SNR of AEP recordings
3. Role of artifact rejection level in enhancing SNR of AEP recordings

2.1 The need for enhancing SNR of AEP recordings

SNR of the recorded waveforms determines the reliability and validity of the inferences drawn from AEPs. It is inevitable to encounter different types of background EEG during AEP recordings and they need to be effectively cut down to ensure good SNR of the AEPs being analyzed. High background EEG can negatively influences the latency, amplitude, morphology and replicability of the waveforms being recorded.

SNR of the recording per se has various applications. The estimated SNR of AEPs is utilized to objectively verify the presence or absence of AEPs, which in turn is used to screen the infants for hearing loss (Elberling & Don, 1987; Don, Elberling & Waring,

1984). It is also helpful in determining whether sub average is of appropriate quality to use as a reference signal in adaptive noise cancellation during AEP recordings (Qiu et al., 1998; Chan et al., 1995). SNR can be an objective measure of comparing AEPs obtained across different background EEG conditions and it serves as a quality measure in comparing different methods used in improving signal acquisition process (Don & Elberling, 1996; Elberling & Don, 1984).

2.2 Some Techniques to Enhance Signal to Noise Ratio (SNR)

In order to effectively cut down the unwanted background activity, it is essential to understand the precise nature and characteristics of the background EEG in terms of its spectral composition, amplitude and the latency. The signal enhancement strategies used in the AEP acquisition primarily focus on one of these parameters. SNR can be improved either by enhancing the evoked potential level or by reducing background noise level or doing both. The current-day AEP equipments have various techniques to enhance the SNR.

2.2.1 Averaging technique

Don and Elberling (1994) reported that averaging technique reduces the background noise level in the final averaged waveform by a factor equal to the square root of the number of sweeps and thereby enhances the SNR. Stecker (2000) reported that during the process of averaging, it cancels out the background EEG which is asynchronous with the stimulus while the AEP which is synchronized with the stimulus persists. Riedel, Granzow and Kollmeier (2001) had studied effect of different averaging methods on the quality of ABR. They used single sweep sorted averaging, weighted

averaging and block weighted averaging methods. They concluded that single sweep sorted averaging resulted in estimation of accurate SNR compared to other methods.

2.2.2 Filtering

In this technique, the unwanted background EEG with frequency content different from frequency content of evoked potentials are filters out. It is reported that filtering technique effectively improves the overall SNR by eliminating the noise components present in the frequency regions where there is presence of very little evoked response or absence of evoked response(Don & Elberling, 1994; Lightfoot & Stevens, 2014). They also reported that when there are overlapping frequency spectra between evoked response and the background noise then filtering offers very limited improvement in SNR of the evoked potential response.

2.2.3 Stimulus parameters

Stimulus repetition rate also improves the SNR. Don and Elberling (1994) reported that lower stimulus repetition rate increases the amplitude of evoked potential response, thereby improving the SNR. They also reported that higher the stimulus level results in better amplitude of AEP, in turn leading to better SNR. Hall(1992) also reported that as the stimulus intensity increases the amplitude of the evoked response increases and becomes higher than the background activities, this in turn improves the SNR and morphology of response waveform.

2.3 Role of Artifact Rejection Level in Enhancing SNR of AEP Recordings

Artifact rejection level is a technique which excludes the unwanted responses from the ongoing average and improves the SNR.ARL is set so that the unwanted potentials whose amplitude is exceeding a determined level of amplitude are rejected and are not included in the averaging process of response waveform of AEPs (Hood, 1998; Hall, 2007; Stecker, 2002).

Maruthy, Gnanateja, Ramachandran and Thuvassery (2015) studied the effect of muscle artifacts on different AEPs in 40 normal healthy adults. They also reported the Minimum Artifact Rejection Threshold (MART) for different artifact conditions (eye blink, teeth clenching, lips spreading, hand stiffness, leg stiffness and neck stiffening) for test protocols suitable for three different AEPs (ABR, MLR &LLR). There results showed that different artifact conditions affect the three different AEPs differently and they also reported that the artifact rejection can be kept at ±20 µV to obtain best AEPs with good SNR. They reported that all the artifact conditions will not affect the AEPs in the same manner.

Sanchez and Gans (2006) studied the effect of two different noise reduction techniques that is Artifact rejection and Bayesian weighting on the wave V amplitude of the ABR during rest and active behavioral conditions. They used two rejection levels of artifact rejection window; one is Artifact rejection equal noise, where the mean artifact rejection level is set at 26 µV and 46 µV for quiet and active ABR conditions, and other is artifact rejection level at ±10µV. The results showed that during rest condition, there was no significant difference between the two different noise reduction techniques but

during active behavioral condition there was a significant reduction in the amplitude of wave V in artifact rejection technique compared to Bayesian weighting technique. So they concluded that strict artifact rejection levels will affect the ABR interpretation.

Don and Elberling (1994) investigated the effect of different artifact rejection levels on ABR recordings. They used seven rejection levels: 10μV, 8.75μV, 7.5μV, 6.25μV, 5μV, 3.75μV and 2.5μV. They found that reducing the artifact rejection window from ±10 μV to ±2.5 μV, reduced the background noise significantly but the overall quality of the response waveform was found to be poor. They also reported that even the SNR did not improve with the reduction in artifact rejection window. Based on their findings, they suggested that appropriate artifact rejection window needs to be set in order to preserve the response amplitude and to remove the background activity.

Pantev and Khvoles (1984) compared the efficiency of three different artifact rejection criteria in recording ABR during quiet and active conditions. There were two different criteria compared; 'level criteria' in which it was checked whether the EEG sweeps increased a particular level in their time function, the 'amplitude criteria' in which frequency of appearance of artifacts is controlled, and the 'power criteria' in which the limit for acceptance and rejection of EEG sweeps were determined based on spectral function. Their results showed that level criteria resulted in best ABR compared to other criteria and suggested that an efficient artifact rejection level need to be set to obtain a reliable ABR in the presence of muscle artifacts.

Lightfoot and Stevens (2014) conducted a study to identify the most efficient artifact rejection level to be used for recording ABR in newborns. They tested ABR at

different artifact rejection levels on 26 newborn babies. The ARL was adjusted sequentially to five different levels (5µV, 6.5µV, 8µV, 10µV & 20µV) and checked the effect of those levels on the test efficiency. The results showed that when the testing conditions are good and the noise level is low, then a strict ARL of 5µV is most efficient, in moderate noise level condition (>30% sweep rejection in 5µV ARL) the 8µV rejection level gave efficient response and in more artifact condition 10µV rejection level gave the highest effectiveness. They concluded that ARL greater than 10µV are more likely to result in poor quality of waveforms.

Ozdamar and Delgado (1996) reported that artifact rejection level is a good method to improve SNR of response. They also reported that when ARL was used, there was an improvement in SNR for high and mid intensity click stimuli, whereas for low level clicks there was no significant improvement in the SNR. This might be due to lack of adequate sweeps required to elicit the response at low levels.

Overall, the review of literature indicates that the studies on the effect of ARL on AEP are not agreement with each other. There is no consensus with respect to the optimum ARL for the acquisition of AEPs. The review of literature warrants more studies in this direction.

CHAPTER-3

METHODS

The study aimed to test the null hypothesis that there is no significant relationship between the different artifact rejection levels (ARLs) and the corresponding AEP recordings. Protocols used in the study conformed to the ethical guidelines for bio-behavioral research involving human subjects set by the All India Institute of Speech and Hearing (Venkatesan, 2009). The details of the method used are given in the subsequent sections.

3.1 Participants

Twenty-three normal hearing, healthy adults in the age range of 18 to 25 years (mean age: 20.13 years) participated in the study. The sample size for the present study was calculated using power test where the effect size (|r|) was 0.25, the power was 0.08 and 'p' was 0.05. All the participants were graduate or post-graduate students at All India Institute of Speech and Hearing.

The normal hearing was ensured using pure tone audiometry, and all the participants had hearing thresholds within 15dBHL at octave frequencies between 250 and 8000Hz. They had type 'A' tympanogram with presence of acoustic reflexes indicating normal middle ear functioning. They had presence of Auditory Brainstem Response (ABR) and Auditory Late Latency Response (ALLR) indicating normal retro cochlear functioning. They had no complaints of past or present otological or neurological problems. All the participants willingly participated in the study and an informed written consent was taken prior to their participation.

3.2 Testing Environment

All the evaluations were carried out in an acoustically and electrically shielded room where the ambient noise levels were within the permissible limits (ANSI S3.1, 1991). Two-room setting was used for pure tone audiometry and single room setting was used for immitance evaluation and auditory evoked potential (AEPs) recordings. AEPs were recorded in the Electrophysiology laboratory of the department of Audiology, All India Institute of Speech and Hearing, Mysuru.

3.3 Instrumentation

A calibrated dual-channel audiometer with standard transducers was used for puretone and speech audiometry. A calibrated GSI-Tympstar Immitance meter was used for evaluating middle ear status. Intelligent Hearing Systems AEP equipment with Smart-EP (version 3.95) software was used for recording ABRs and ALLRs.

3.4 Test Procedure

3.4.1 Candidacy assessment

Candidacy assessment included a structured interview, puretone audiometry and immitance evaluation. The structured interview probed into the participant's demographic details, auditory abilities, past as well as presents otological and neurological functioning and their willingness to participate in the study. Ear-specific puretone thresholds were estimated between 250Hz and 8kHz using modified Hughson and Westlake method (Carhart & Jerger, 1959). Speech recognition threshold was assessed using the standardized spondee word list or paired-word list available in participants' respective language. The spondees or paired-word lists were delivered through the Telephonic ear

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TDH-39 head phones and speech recognition threshold was estimated in 5 dB steps using bracketing method. The speech identification scores were assessed at 40dBSL (ref: Speech Recognition Threshold).

Tympanogram was measured for both the ears using a probe tone of 226Hz and by sweeping the ear canal pressure from +200daPa to -400daPa. Static admittance, tympanometric peak pressure and the equivalent ear canal volume were noted down from each tympanogram. Both ipsilateral and contralateral acoustic reflexes were measured for puretones of 500Hz, 1kHz, 2kHz and 4kHz.

3.4.2 Experimental test procedure

This involved recording of AEPs using different ARL. To begin with, the participants were explained the purpose and protocol of the study. Participants were comfortably seated in a reclining chair. For both ABR and ALLR, the noninverting electrode was placed at Cz (vertex), inverting electrode was placed at M2 and the ground electrode was placed at M1 of the scalp, according to the international 10-20 system (Jasper, 1958). The electrode sites were cleaned with a Nuprep gel, following which silver chloride electrodes were firmly placed using adequate amount of Ten-20 conductive paste and an adhesive tape. It was ensured that the inter-electrode impedance is <2 kΩ and the absolute electrode impedance is <5 kΩ throughout the testing time.

Participants were instructed to stay relaxed and minimize extraneous movements such as eye blinking, swallowing, hand, leg and head movements during the recording. The protocol used to record ABR and ALLR are shown in Table 3.1. The responses were recorded for the right ear stimulation at 80dBnHL through ER-3A insert earphones. The

parameter of interest was the ARL expressed in µV in Intelligent Hearing Systems (IHS). ABRs and ALLRs were recorded by setting the ARL at four different levels, while all other parameters remained same.

In all the participants, ABRs were recorded first and then the ALLRs were recorded. To begin with, ABRs were recorded by keeping the ARL at Minimum Artifact Rejection Threshold (MART) which was estimated using the method given by Maruthy Gnanateja, Ramachandran and Thuvassery (2015). In each recording, the online EEG was monitored to determine the MART (in µV) such that an average response of 2000 sweeps could be recorded with less than 10% sweep rejections. This was considered as the MART level. Then the ABRs were recorded at three other ARL (20µV, 30µV & 40µV) as mentioned in the Table 3.1. At each ARL, ABRs were recorded twice to ensure replicability of the response waveforms.

Similarly, ALLRs were recorded first at individual MART level and then at three other ARLs mentioned in Table 3.1 (25µV, 50µV & 75µV). At each ARL, ALLRs were recorded twice to ensure replicability of the response waveforms.

3.5 Response Analysis

3.5.1 Auditory brainstem responses

After ensuring the replicability of waveforms at each ARL, wave I, III and V (Jewett, 1971) were marked by two audiologists, experienced in the field of electrophysiology. Peak latency and peak amplitude of the measured waves were noted down from one of the recordings. The amplitude of the waves was determined as the difference in the amplitude between peak and the following trough of the wave.

Table 3.1: Stimulus and acquisition parameters used for recording ABR and ALLR in the present study

	ABR	**ALLR**
Stimulus Parameters		
Stimuli	Click	Syllable /da/
Duration	100µs	100ms
Intensity level	80dBnHL	80dBnHL
Transducer	ER3A insert ear phone	ER3A insert ear phone
Polarity	Rarefaction	Rarefaction
Rate of presentation	30.1/s	1.1/s
No. of sweeps	2000	150
Acquisition Parameters		
Number of channels	Single	Single
Recording Epoch	15ms	600ms
Filter	100-3000Hz	1-30Hz
Notch filter	Off	Off
Electrode montage	Vertical	Vertical
Mode of presentation	Ipsilateral	Ipsilateral
Amplification	1,00,000	1,00,000
Artifact rejection level	MART, 20µV, 30µV & 40µV	MART, 25µV, 50µV & 75µV

***Note**: MART- Minimum Artifact Rejection Level*

Figure 3.1 shows marking of the waves in a sample waveform. Replicability of ABR was objectively estimated using wave-wave correlation and correlation coefficient was derived. Apart from the latency, amplitude and replicability, SNR of the averaged waveform was also determined as a target measure. SNR was determined using the default algorithm of Smart EP software. The algorithm uses split-sweep method and calculates SNR using below mentioned formula. The SNR of ABR was determined for the latency region of 0 ms to 6.5 ms.

$$SNR = (A+B) / (A-B)$$

Wherein, A refers to Peak-peak amplitude of one buffer, and B refers to Peak-peak amplitude of another buffer.
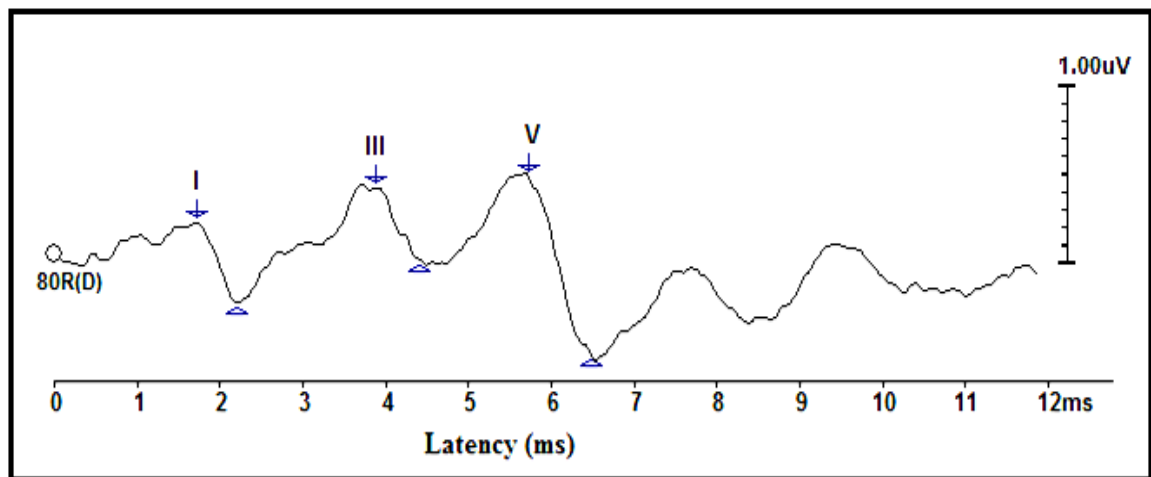
Figure 3.1: Sample ABR waveform showing marking of waves.

3.5.2 Auditory late latency response

After ensuring the replicability of waveforms, ALLRs were visually analyzed by the same experts (experts who analyzed ABR) to mark P_1, N_1 and P_2. The peak latency of P_1, N_1 and P_2, and their corresponding peak-peak amplitudes (P_1-N_1 & N_1-P_2) were noted

down from the marked waveforms. Figure 3.2 shows the marking of waves in a sample ALLR waveform. Replicability of ALLR was objectively estimated using wave-wave correlation, and the corresponding correlation coefficient was derived. The SNR was determined using the same procedure of that of ABR. However in ALLR, the SNR was determined for the latency region of 30ms to 300ms.
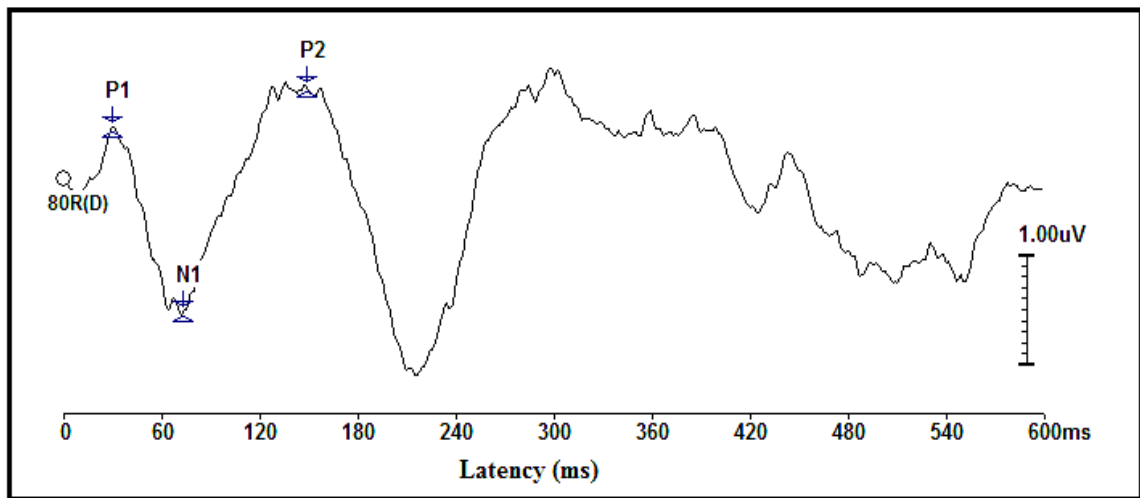
Figure 3.2: Sample ALLR waveform showing marking of waves.

CHAPTER-4

RESULTS

The present study aimed to test the effect of different artifact rejection levels (ARL) on Auditory Brainstem Responses (ABR) and Auditory Late Latency Responses (ALLR). In the present study, the ARL (four different levels) was the independent variable, whereas measures of ABR and ALLR (latency, amplitude, correlation coefficient of the two replications & SNR) were the dependent variables.

The data were initially tested for their normal distribution using Shapiro-Wilks test of normality. There were 28 variables in ABR and 23 variables in ALLR. The results of the test (given in Appendix I) showed that all the variables of ABR were normally distributed while some of the variables of ALLR were not. Hence a parametric test (Repeated measures ANOVA) was used for assessing the effect of ARL on the measures of ABR. Whereas, nonparametric test (Friedman test) was used to assess the effect of ARL on the measures of ALLR. The results obtained in the present study are reported under following broad headings;

1. Effect of different artifact rejection level on ABR
2. Effect of different artifact rejection level on ALLR

4.1 Effect of Different Artifact Rejection Level on ABR

4.1.1 Effect on latency of ABR

The mean and standard deviation (SD) of latency of wave I, III and V of ABR obtained in the four different ARLs are given Table 4.1. Mean and standard deviation of

MART in ABR is 9.13µV & 0.34.There was no common pattern in the way the mean latency varied across the ARLs. The results of repeated measures ANOVA showed that there was no significant effect of ARL on the latency of wave I [F (3, 66) = 0.16, p = 0.91], III [F(3, 66) = 0.07, p = 0.68] and V [F(3, 66) = 0.005, p = 0.99].

Table 4.1: Mean and standard deviation (SD) of latency of wave I, III and V of ABR obtained in the four artifact rejection levels

Wave	Statistical Measure	Artifact Rejection Level			
		MART	20 µV	30 µV	40 µV
I	Mean (ms)	1.68	1.68	1.69	1.68
	SD	0.14	0.14	0.14	0.14
III	Mean (ms)	3.77	3.76	3.75	3.76
	SD	0.10	0.10	0.10	0.16
V	Mean (ms)	5.59	5.63	5.61	5.60
	SD	0.15	0.13	0.14	0.13

Note: MART- Minimum Artifact Rejection Threshold

4.1.2 Effect on amplitude of ABR

The mean and standard deviation (SD) of amplitude of wave I, III and V of ABR obtained in the four different ARLs (MART, 20µV, 30µV & 40µV) are given in Table 4.2. There was no common pattern in the way the mean amplitudes varied across the ARLs. The results of Repeated measures ANOVA showed that there was no significant

main effect of ARL on the amplitude of wave I [F(3, 66) = 0.99, p= 0.4], III [F(3, 66) = 0.78, p= 0.50] and V [F(3, 66) = 0.14, p= 0.93].

Table 4.2: Mean and standard deviation (SD) of amplitude of wave I, III and V of ABR obtained in the four artifact rejection levels

Wave	Statistical Measure	Artifact Rejection Level			
		MART	20 µV	30 µV	40 µV
I	Mean (µV)	0.30	0.29	0.32	0.31
	SD	0.11	0.10	0.13	0.13
III	Mean (µV)	0.30	0.32	0.32	0.30
	SD	0.11	0.10	0.09	0.10
V	Mean (µV)	0.62	0.61	0.62	0.62
	SD	0.15	0.16	0.15	0.14

Note: *MART- Minimum Artifact Rejection Threshold*

4.1.3 Effect on replicability of ABR

The mean and standard deviation (SD) of correlation coefficient of replicability of ABR obtained in the four different ARLs (MART, 20µV, 30µV & 40µV) are given in Table 4.3. The results of Repeated measures ANOVA showed that there was no significant main effect of ARL on replicability [F (3, 66) = 0.98, p= 0.40] of ABR.

Table 4.3: Mean and standard deviation (SD) of correlation coefficient of replicability of

ABR obtained in the four artifact rejection levels

Statistical Measure	Artifact Rejection Level			
	MART	20 µV	30 µV	40 µV
Mean	0.90	0.88	0.87	0.88
SD	0.04	0.08	0.10	0.07

Note: *MART- Minimum Artifact Rejection Threshold*

4.1.4 Effect on SNR of ABR

The mean and standard deviation (SD) of SNR of ABR obtained in the four different ARLs (MART, 20µV, 30µV & 40µV) are given in Table 4.4. The results of Repeated measures ANOVA showed that there was no significant main effect of ARL on SNR [F (3, 66) = 2.74, p= 0.05] of ABR. Figure 4.1 shows the grand averaged waveform of 46 waveforms of the ABR obtained in the four ARLs. The figure shows that ABR recorded appears similar across the four ARLs.

Table 4.4: Mean and standard deviation (SD) of SNR of ABR obtained at four different artifact rejection levels

Statistical Measure	Artifact Rejection Level			
	MART	20 μV	30 μV	40 μV
Mean (dB)	1.96	2.32	2.08	1.94
SD	0.80	0.92	0.60	0.61

Note: *MART- Minimum Artifact Rejection Threshold*
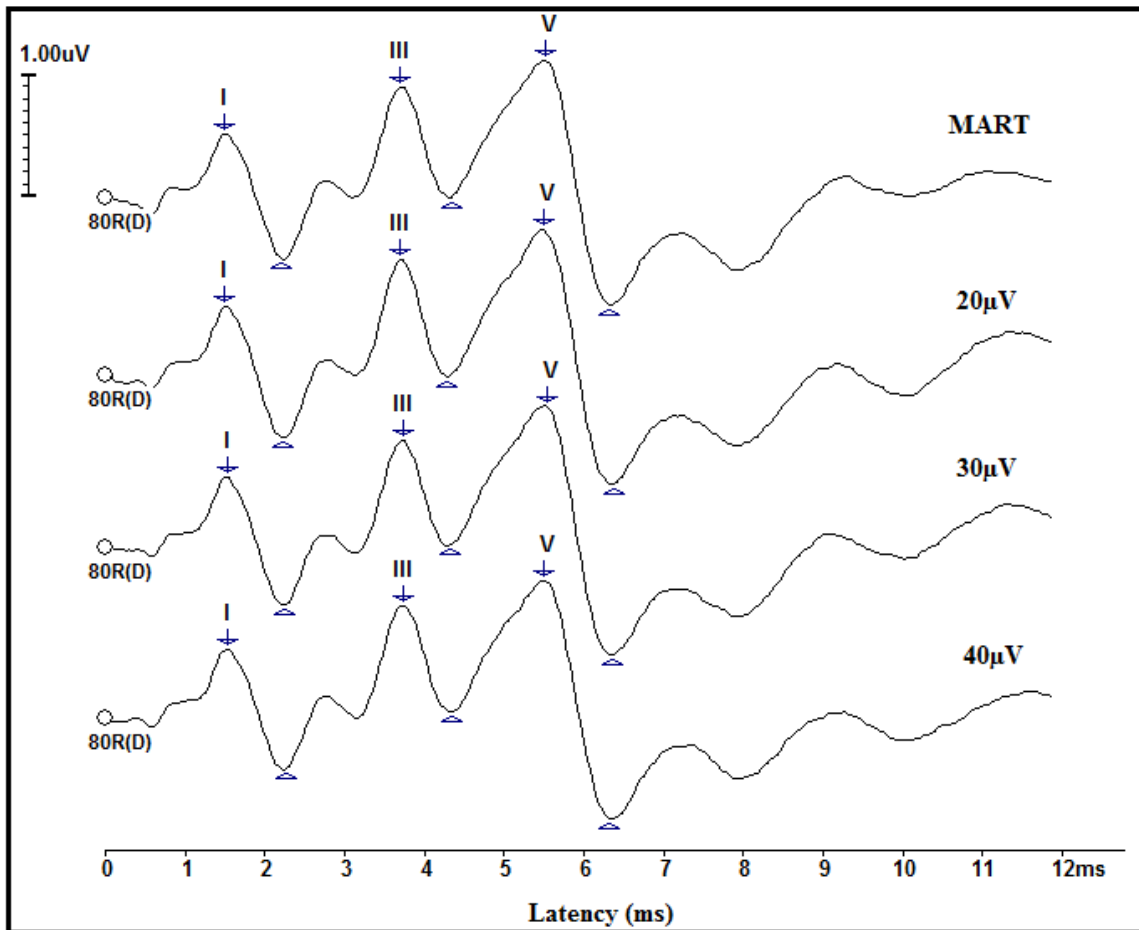
Figure 4.1: Grand averaged waveforms of 46 waveforms of the ABR obtained in the four different artifact rejection levels.

4.2 Effect of Different Artifact Rejection Level on ALLR

4.2.1 Effect on latency of ALLR

The median and interquartile range of peak latency of ALLRs obtained at the four different ARLs (MART, 25µV, 50µV & 75µV) are given in Table 4.5. Mean and standard deviation of MART in ALLR is 22.95µV & 0.36. The results of Friedman test showed that there was no significant effect of ARL on the latency of P_1 [$\chi^2(3) = 3.21$, p = 0.35], N_1 [$\chi^2(3) = 1.50$, p = 0.68] and P_2 [$\chi^2(3) = 6.41$, p = 0.09] of ALLR.

Table 4.5: Median and interquartile range of peak latency of ALLRs obtained at four different artifact rejection levels

Wave	Statistical Measure	Artifact Rejection Level			
		MART	25 µV	50 µV	75 µV
P_1	Median (ms)	38.4	39.6	39.6	37.2
	Interquartile range	12	9.6	13.8	16.5
N_1	Median (ms)	103	102	103	102
	Interquartile range	18	20.4	12	16.8
P_2	Median (ms)	153	157	156	158
	Interquartile range	12	10.8	9.6	14.4

Note: MART- Minimum Artifact Rejection Threshold

4.2.2 Effect on amplitude of ALLR

The median and interquartile range of amplitude of ALLRs obtained in four different ARLs (MART, 25µV, 50µV & 75µV) are given in Table 4.6. The results of Friedman test showed that there was no significant effect of ARL on the amplitude of P_1 - N_1 [χ^2 (3) = 4.72, p = 0.19] and N_1 - P_2 [χ^2 (3) = 7.40, p = 0.06] of ALLR.

Table 4.6: *Median and interquartile range of peak to peak amplitudes of ALLRs obtained in four different artifact rejection levels*

Wave complex	Statistical Measure	Artifact Rejection Level			
		MART	25 µV	50 µV	75 µV
P_1 - N_1	Median (µV)	1.82	1.95	2.35	2.24
	Interquartile range	0.99	1.31	1.71	1.27
N_1 - P_2	Median (µV)	2.58	2.49	3.09	2.72
	Interquartile range	1.65	2.29	2.44	1.81

Note: MART- Minimum Artifact Rejection Threshold

4.2.3 Effect on replicability of ALLR

The median and interquartile range of correlation coefficient of replicability of ALLRs obtained using the four different ARLs are given in Table 4.7. The results of Friedman test showed that there was no significant effect of ARL on the replicability of ALLR [χ^2 (3) = 4.33, p = 0.22].

Table 4.7: Median and interquartile range of correlation coefficient of replicability of ALLRs obtained in four different artifact rejection levels

Statistical Measure	Artifact Rejection Level			
	MART	25 µV	50 µV	75 µV
Median	0.77	0.82	0.81	0.78
Interquartile range	0.14	0.15	0.23	0.17

Note: MART- Minimum Artifact Rejection Threshold

4.2.4 Effect on SNR of ALLR

The median and interquartile range of SNR of ALLRs obtained using the four different ARLs are given in Table 4.8. The results of Friedman test showed that there was a significant effect of ARL on SNR of ALLR [χ^2 (3) = 10.261, p = 0.01].

Subsequently, pair-wise comparison of the SNR across the ARLs was made using Wilcoxon singed rank test and the results revealed that the SNR with 25µV was significantly higher than that of MART (Z = -2.06, p = 0.03, |r| = 0.62) and, SNR with 50µV ARL was significantly higher than that of MART (Z = -2.64, p = 0.008, |r| = 0.35). There was no significant difference in SNR between MART and 75µV (Z = -1.397, p = 0.16), 25µV and 50µV (Z = -0.06, p = 0.95), 25µV and 75µV (Z = -0.38, p = 0.70) and, 50µV and 75µV (Z = -1.02, p = 0.30). Figure 4.2 shows the grand average of 46 waveforms of the ALLRs obtained in the four different ARLs. Overall, the waves appear similar across the four ARLs.

Table 4.8: Median and interquartile range of SNR of ALLRs obtained in four different artifact rejection levels

Statistical Measure	Artifact Rejection Level			
	MART	25 µV	50 µV	75 µV
Median	1.33	1.44	1.5	1.43
Interquartile range	0.43	0.68	0.43	0.31

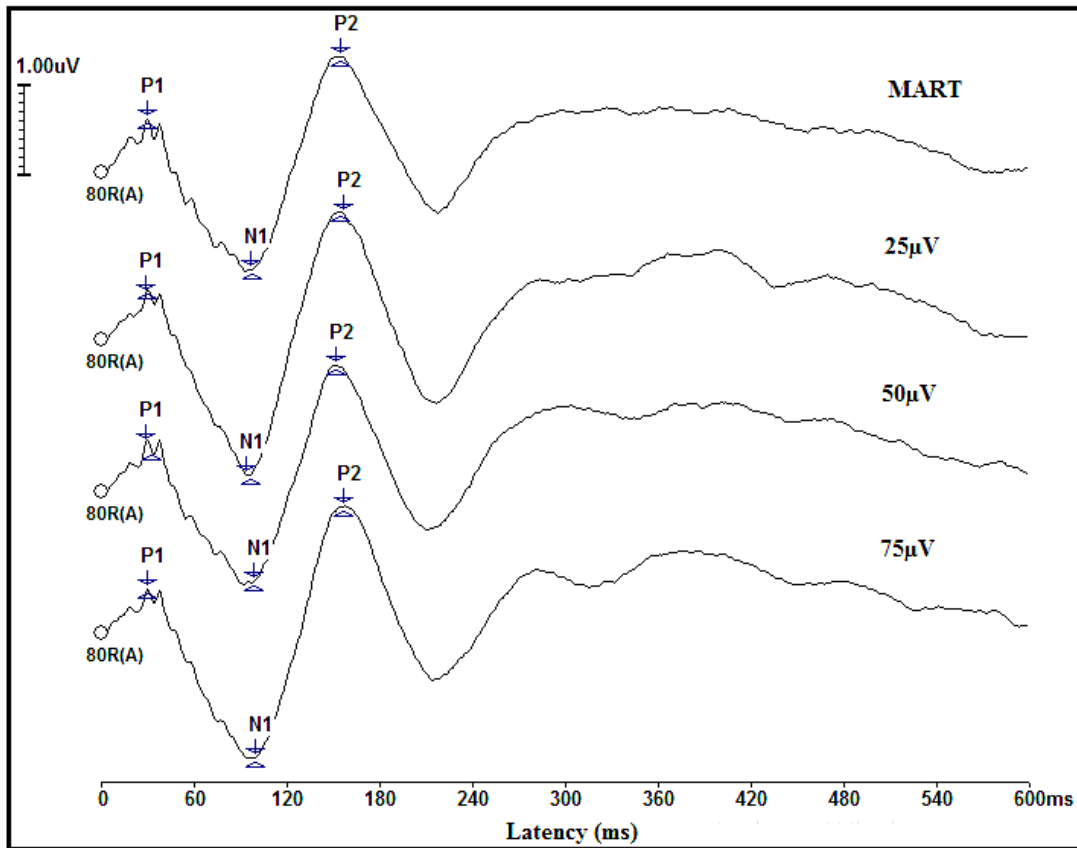***Note:*** *MART- Minimum Artifact Rejection Threshold*

Figure 4.2: Grand averages of 46 waveforms of the ALLR obtained at four different artifact rejection levels.

CHAPTER-5

DISCUSSION

The aim of the present study was to identify the optimal artifact rejection level (ARL) to record Auditory Brainstem Responses (ABRs) and Auditory Long Latency Responses (ALLRs). ABRs and ALLRs were recorded in four different ARLs and their measures (latency, amplitude, replicability & SNR) were analyzed for the differences, if any. Overall, the results of the present study revealed no significant influence of different ARLs on the measures of ABR while the significant influence of it was present only on the SNR of ALLR. The results are discussed in light of their basis, clinical relevance, technical reasoning and their significance with reference to the earlier studies under the following broad headings;

1. Influence of artifact rejection level on ABR
2. Influence of artifact rejection level on ALLR

5.1 Influence of Artifact Rejection Level on ABR

Conventionally, the ARL is varied based on the auditory evoked potentials recorded. For ABR, ARLs are set around ±25 µV and for ALLR around ±50 µV. The background EEG activity is likely to vary across individuals (Maruthy, Gnanateja, Ramachandran & Thuvassery, 2015), which means that the ARL cannot be set at a constant level across individuals. Maruthy et al. (2015) showed that Minimum Artifact Rejection Threshold (MART) can be as low as 10µV in some of the individuals. Based on their findings they suggested that, higher ARL is likely to include higher levels of

background EEG resulting in averaged responses with poorer SNR. Therefore, the present study intended to scientifically verify their assumption by recording averaged ABRs and ALLRs across various ARLs.

The results of ABR revealed that setting different ARLs did not show any significant change in ABR. This was true with all the measures of ABR. The finding refutes the assumption of Maruthy et al. (2015). Latency, amplitude and replicability of AEPs are the key parameters considered for clinical use, both in threshold estimation and site of lesion testing. All three measures were found be comparable across the four ARLs used in the study (MART, 20µV, 30µV & 40µV). This suggests that, for all clinical purposes, ARL can be set up to 40µV and one need not estimate individual MART, as recommended by Maruthy et al. (2015).

The variations in the ARLs are expected to accept different levels of background activity during averaging and it is possible that such different levels of background EEG up to 40µV do not influence the latency, amplitude and replicability of ABRs significantly. Therefore, it was important to analyze whether the similarity in these measures is true due to the similar SNR across the four ARLs or holds true even if the SNRs of the waves are different. The study revealed that SNR did not vary across ARLs. This suggests that levels of background EEG remains same up to 40µV and the similarity in latency, amplitude and replicability of ABRs is attributable to the similar SNRs of the waveforms.

Maruthy et al. (2015) had reported that ABR is affected strongly by teeth clenching followed by neck stiffening and, hand and leg movements. Sanchez and Gans

(2005) also reported that during minimal muscle interference condition there was no effect of ARL on the amplitude of ABR. But, there was a significant reduction in the ABR amplitude across ARLs during extraneous movements like jaw movement and head movement. Lightfoot and Stevens (2013) reported that during low levels of background EEG activity, the ARL of 5µV was adequate whereas, when the background EEG level increased, the higher ARL was most efficient. These muscle interferences were minimized in the present study by instructing the participants to avoid extraneous movement and to stay relaxed and calm during the recording. The absence of significant changes in ABR across the four ARLs should be interpreted in light of the relaxed state of the participants. The results may not hold true in instances of significant muscle interferences, wherein the SNR of the EEG picked up at the electrode is likely to be poor. Future studies can tap the effect of ARLs in instances of significant muscle interferences.

In the study, ABRs were recorded for high intensity stimulation (80dBnHL). Whether the findings can be generalized to ABRs recorded at threshold levels is a debatable issue. It is important to note that there was no significant change in latency and amplitude of wave I of ABR. Wave I being a low amplitude response remained stable across the four ARLs. Based on this one can infer that wave V recorded at lower intensity (with lesser amplitude) is likely to remain unchanged across the four ARLs. Future studies can attempt to validate this inference by recording ABRs at lower intensities.

In the study ABR was measured at MART level which was as low as 9µV and the ABR recorded at MART did not significantly differ from that of 40µV. Therefore, one need not estimate MART to decide the ARL, rather can set to any value up to 40µV.

5.2 Influence of Artifact Rejection Level on ALLR

ALLR is a clinically important auditory evoked potential, useful for both hearing threshold estimation and neurodiagnosis. It is important for clinicians and researchers alike. Therefore, it was of interest of the present study to determine the effect of ARL on ALLR and identify the most appropriate ARL for a good recording. It is important to note that the findings obtained in ABR cannot be generalized to ALLR, as the nature of ALLR (in terms of frequency, amplitude & latency of neurons involved) as well the interfering muscle potentials are different compared to ABR.

The results of ALLR revealed that setting different ARLs did not result in significant change in latency, amplitude and replicability of the ALLR. This means that ALLRs for clinical purposes can be recorded with any ARL up to 75μV and it assures no change in the clinical interpretations. Maruthy et al. (2015) reported that eye blink was the major source of interference on ALLR response and recommended use of a separate channel to record eye blink in order to eliminate them. In the present study, no attempt was made to eliminate the eye blinks by using a separate channel. Yet, the measures of ALLR remained unchanged across all the four ARLs. Lower ARLs are likely to eliminate the interference of eye blinks while the higher ARLs are expected to accept them for averaging. The absence of significant difference across the ARLs in their latency, amplitude and replicability suggests that differences in the interference if any, does not alter these clinical measures.

The different ARLs are expected to include different levels of background activity during averaging and it is possible that such different levels of background EEG activity

influences the SNR of the averaged waveform, but do not influence the latency, amplitude and replicability of ALLRs. Therefore, it was important to analyze whether the similarity in these measures is true even if the SNRs of the waveforms are different. The study revealed that SNR significantly varied across ARLs. This suggests that even when the SNR varied across different ARLs, the latency, amplitude and replicability remained similar across ARLs up to 75µV.

In the study, MART for ALLR was as low as 23µV and at this ARL, the mean SNR was lower compared to that of 25µV and 50µV. This may be due to the rejection of few ALLRs at lower ARL which may result in reduced SNR. Maruthy et al. (2015) had reported that if the ARLs are increased to higher level then there are high chances of background activity getting averaged with the response waveform resulting in poorer SNR. However, the current results do not support their notion as it showed that 25µV and 50µV ARL resulted in higher SNR than the ARL being set at MART.

Interestingly, it was found in the study that the SNR of ALLR waveform was comparable between MART and 75µV. The SNR at 75µV was also similar to that of 25µV and 50µV. This suggests that SNR has a tendency to decrease with increase in the ARL beyond 50µV. This may be due to addition of background activity to the averaged response waveform if ARL is set to 75µV. Future studies can tap this issue by taking ARLs higher than 75µV.

For clinical utility, an attempt was made in the present study to determine an optimum ARL for ABR and ALLR. The findings suggest that any ARL up to 40µV can be used for recording ABR with good SNR and it is not necessary to estimate MART to

decide the ARL. However in ALLR, the results are suggestive of use of 25µV or 50µV

ARL for recordings with good SNRs.

CHAPTER-6

SUMMARY AND CONCLUSIONS

Optimizing the stimulus and recording parameters is crucial in obtaining high quality waveforms of auditory evoked potentials (AEPs). The biggest challenge in far-field recording of AEPs is to minimize the interference of background EEG and enhance the SNR of averaged waveforms. The present study aimed to systematically investigate the effect of artifact rejection levels (ARLs) on auditory brainstem responses (ABRs) and auditory late latency responses (ALLRs) with an attempt to identify the most appropriate ARL for the two highly applied AEPs in the clinical audiology.

Twenty-three normal hearing individuals in the age range of 18 to 25 years (mean age: 20.13 years) participated in the study. In these participants, ABR was recorded in four different ARLs (MART, 20µV, 30µV & 40µV) and the ALLR was also recorded in four different ARLs (MART, 25µV, 50µV & 75µV). Latency, amplitude, replicability and SNR were measured from the averaged waveforms and were compared across the four ARLs. Based on the results of normality test, repeated measures ANOVA was used to statistically assess the effect of ARL on measures ABR, and Friedman test was used to assess the effect of it on the measures ALLR.

Results revealed that there was no significant effect of different ARLs on ABR. This was true with all the four measures of ABR. Whereas in ALLR, there was a significant effect of ARL on the SNR of waveforms. Pair-wise comparison of the SNR across the ARLs was made using Wilcoxon singed rank test and the results revealed that

the SNR with 25µV and 50µV ARL was significantly higher than that of MART. SNR at 75µV was comparable to the SNR recorded in the other three ARLs.

From the findings of the present study, it can be concluded that there is no significant influence of ARL on the measures of ABR up to 40µV and one can record ABR with good SNR up to 40µV. In case of ALLR, it is recommended to use ARL of either 25µV or 50µV for recordings with good SNR.

In the present study, ABRs and ALLRs were measured when the participants were relaxed and refrained from body movements. Therefore, it is suggested to restrict the findings to the recordings with minimal muscle interference. In instances of muscle interference, there may be variations in the measures of ABR and ALLR across different ARLs. Future studies can tap the effect of ARL on AEPs recorded during significant muscle activities. Attempts can be also made to assess the effect of ARL on AEPs recorded at threshold levels.

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APPENDIX–1

Results of Shapiro-Wilk test of normality for the measures of ABR and ALLR (df=23)

ABR		ALLR	
Measure	p	Measure	p
Amplitude I MART	0.30	P_1 latency MART	0.01
Amplitude I 20μV	0.90	N_1 latency MART	0.85
Amplitude I 30μV	0.55	P_2 latency MART	0.09
Amplitude I 40μV	0.38	P_1 latency 25μV	0.03
Amplitude III MART	0.24	N_1 latency 25μV	0.26
Amplitude III 20 μV	0.34	P_2 latency 25μV	0.60
Amplitude III 30μV	0.51	P_1 latency 50μV	0.03
Amplitude III 40μV	0.79	N_1 latency 50μV	0.39
Amplitude V MART	0.78	P_2 latency 50μV	0.00
Amplitude V 20μV	0.54	P_1 latency 75μV	0.01
Amplitude V 30μV	0.51	N_1 latency 75μV	0.69
Amplitude V 40μV	0.40	P_2 latency 75μV	0.03
Latency I MART	0.68	P_1N_1 amplitude MART	0.00
Latency I 20μV	0.18	P_1N_1 amplitude 25μV	0.00
Latency I 30μV	0.33	P_1N_1 amplitude 50μV	0.00
Latency I 40μV	0.53	P_1N_1 amplitude 75 μV	0.00
Latency III MART	0.64	N_1P_2 amplitude MART	0.03
Latency III 20μV	0.41	N_1P_2 amplitude 25μV	0.00

ABR		ALLR	
Measure	p	Measure	p
Latency III 30μV	0.46	N_1P_2 amplitude 50μV	0.01
Latency III 40μV	0.00	N_1P_2 amplitude 75μV	0.00
Latency V MART	0.86	SNR MART	0.68
Latency V 20μV	0.45	SNR 25μV	0.21
Latency V 30μV	0.16	SNR 50μV	0.13
Latency V 40μV	0.76	SNR 75μV	0.01
SNR MART	0.03	Replicability MART	0.96
SNR 20μV	0.27	Replicability 25μV	0.31
SNR 30μV	0.64	Replicability 50μV	0.02
SNR 40μV	0.17	Replicability 75μV	0.13
Replicability MART	0.02		
Replicability 20μV	0.00		
Replicability 30μV	0.00		
Replicability 40μV	0.01		