Frequency Following Responses in Individuals with Auditory Neuropathy Spectrum Disorder

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(Audiology)

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JUNE, 2026

CERTIFICATE

This is to certify that this dissertation entitled **"Frequency Following Responses in Individuals with Auditory Neuropathy Spectrum Disorder"** is a bonafide work submitted as part of fulfilment of the degree of Master of Science (Audiology) of the student with Registration Number **P01II24S023002**. 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 **"Frequency Following Responses in Individuals with Auditory Neuropathy Spectrum Disorder"** has been prepared under my supervision and guidance. It is also being 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 **"Frequency Following Responses in Individuals with Auditory Neuropathy Spectrum Disorder"** is the result of my own study under the guidance of Dr. Ajith Kumar U., Professor 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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ABSTRACT

Auditory neuropathy is a disorder characterized by normal outer hair cell function and dys-synchrony of the auditory nerve, resulting in difficulty understanding speech in noise. The frequency following response (FFR), a measure of phase locked subcortical brain activity, which offers an objective means of quantifying temporal coding deficits in this population. The current study aimed to compare the spectral amplitude of FFR in response to sinusoidal amplitude modulated (SAM) tones and the speech syllable /ba/ between individuals with AN & those with normal hearing (NL).

Thirty participants with the age range of 16 – 40years were recruited for the study. FFRs were recorded using horizontal electrode montage with gold foil tip trodes at 80dB nHL. The responses were elicited using 500 Hz SAM pure tone 100% modulated at 16 Hz, 32 Hz & 64 Hz and 170 ms synthetic /ba/ syllable. FFR amplitudes at the modulation frequencies (for SAM stimuli) and at the fundamental frequencies (for speech stimuli) were obtained from the envelope waveforms generated by adding the FFR responses to condensation and rarefaction stimuli. The envelope amplitudes (FFR_{ENV}) were extracted using the Fast Fourier Transform (FFT) in matrix laboratory (MATLAB) and were analysed using the generalized linear mixed model (GLMM) and Mann–Whitney U tests.

FFR_{ENV} amplitudes were significantly lower in the AN group at 32 Hz and 64 Hz modulation frequencies, while amplitudes at 16 Hz were comparable between the two groups. In both groups, FFR_{ENV} amplitude decreased with increasing modulation frequency. For the /ba/ syllable, the AN group demonstrated significantly lower FFR_{ENV} amplitudes than the NL group. The findings confirm that individuals with AN exhibit significant deficits in subcortical temporal envelope encoding, particularly for faster modulation rates and speech

signals, consistent with disrupted auditory nerve synchrony. The relative preservation of responses at 16 Hz suggests residual cortical envelope processing in AN.

CHAPTER 1

INTRODUCTION

Auditory neuropathy (AN) disrupts the phase-locking of action potentials in the auditory nerve, resulting in dys-synchronous neural firing. Clinically, individuals with AN exhibit normal otoacoustic emissions (OAE) and/or cochlear microphonics, while acoustic reflexes and auditory brainstem responses (ABR) are absent or abnormal (Berlin et al., 2003; Starr et al., 1996). Speech perception in AN is typically disproportionately poor relative to the degree of hearing loss, and hearing aids provide limited or no benefit. Etiologies of AN can be syndromic as well as non-syndromic, environmental causes, or structural anomalies. It can be prenatal, including genetic cochlear malformation; fetal infection, such as measles, mumps, or cytomegalovirus; dysmaturity; or postnatal, such as prematurity, perinatal disorders such as severe icterus and kernicterus, hypoxia with mechanical ventilation, septicaemia, ototoxic drugs, or meningitis (De Siati et al., 2020; Riggs et al., 2017). The pathophysiology involves dysfunction at multiple sites, including presynaptic disorders affecting inner hair cells and ribbon synapses, postsynaptic disorders affecting unmyelinated auditory nerve dendrites, auditory ganglion cells with their myelinated axons, and central auditory brainstem pathways (Rance & Starr, 2015).

Temporal coding refers to the precise timing of auditory nerve spikes representing both slow envelopes and the rapid fine structure of sounds. In normal hearing (NL), auditory nerve fibres phase-lock to these cues, supporting pitch and speech perception. In AN, this neural synchrony is disrupted; patients often show normal outer hair-cell function but abnormal neural responses (absent or distorted ABRs). Human studies confirm deficits in both envelope and fine-structure coding. For instance, AN listeners required greater modulation depths to detect amplitude modulation (AM) and frequency modulation (FM) (Narne, 2013), and observed absent frequency-following responses (FFRs) and severe

speech-in-noise difficulties (White-Schwoch et al., 2022). These findings demonstrate that impaired temporal coding in the auditory nerve underlies the speech perception problems in AN.

In line with these findings, modulation detection studies further highlight temporal coding deficits in AN. Listeners with AN required significantly higher depths to detect both AM and FM, indicating poor encoding of temporal envelope and fine-structure cues (V. K. Narne, 2013). Similarly, individuals with AN had elevated AM detection threshold, particularly at higher modulation rates, reflecting impaired neural synchrony in processing rapid temporal changes (Yuvaraj & Jayaram, 2016). These results reinforce that abnormal modulation detection is a hallmark of AN and provides a behavioural correlate of disrupted temporal coding at the auditory nerve level.

Need for the study

AN is a complex auditory disorder characterized by disrupted neural synchrony despite normal cochlear outer hair cell function (Starr et al., 1996). Clinically, individuals with AN present with disproportionate difficulties in speech perception relative to their pure tone audiometry thresholds, particularly in noisy environments (Rance & Starr, 2015).

It is hypothesized that abnormal phase-locking in the auditory pathway is responsible for the disproportionately poor speech understanding abilities observed in individuals with AN. The human FFR represents the phase-locked neural activity of the auditory system to both the envelope and the temporal fine structure of acoustic stimuli. Investigating the encoding of the envelope and temporal fine structure in individuals with AN may provide an objective index of the severity of the phase-locking abnormality in this population.

Aim of the study

The aim of the present study is to compare the spectral amplitude of FFR in response to sinusoidal amplitude-modulated (SAM) tones and speech sounds between individuals with AN and those with NL.

Objectives

1. To compare the spectral amplitude of FFR in response to a 500 Hz pure tone SAM at 16 Hz, 32 Hz, and 64 Hz between individuals with AN and those with NL.
2. To compare the spectral amplitude of FFR across different modulation frequencies within each group (AN and NL)
3. To compare the spectral amplitude of FFR in response to a /ba/ syllable between individuals with AN and those with NL.

Null Hypotheses

1. There is no significant difference between the spectral amplitude of FFR in response to a 500 Hz pure tone SAM at 16 Hz, 32 Hz and 64 Hz between individuals with AN and those with NL.
2. There is no significant difference between the spectral amplitude of FFR across different modulation frequencies within each group (AN and NL).
3. There is no significant difference between spectral amplitude of FFR in response to a /ba/ syllable between individuals with AN and those with NL.

CHAPTER 2

REVIEW OF LITERATURE

Sinusoidal amplitude-modulated (SAM) tones are commonly used to evoke the frequency-following response (FFR) because they enable researchers to manipulate the temporal envelope rate independently of the carrier frequency. In listeners with normal hearing (NL), strong FFR_{ENV} can be recorded across a wide range of modulation frequencies. It was shown that neurons within the inferior colliculus phase-lock effectively to the modulation envelope of amplitude-modulated tones (Batra et al., 1989). However, the response strength decreases as modulation frequency increases, which is believed to reflect the upper limits of neural phase-locking within the ascending auditory system.

Similarly, FFRs were elicited by SAM tones in adults with NL (Purcell et al., 2004). Their findings revealed that spectral amplitudes at the modulation frequency were strongest at lower modulation rates (16–32 Hz) and gradually reduced at higher rates (64–128 Hz). This trend has been consistently reported in subsequent studies and is generally interpreted as evidence of the limited temporal processing capacity of subcortical auditory pathways rather than an effect of the stimulus itself. In addition, FFR_{Env} responses in NL individuals are highly stable across recording sessions and are sensitive to subtle auditory changes, highlighting their potential clinical value (Picton et al., 2003)

Apart from response amplitude, the phase characteristics of the FFR_{Env} also provide important information about auditory processing. The phase relationship between the neural response and the modulation waveform, often referred to as the modulation-following response phase, changes systematically with modulation frequency. Studies showed that phase delays increase as modulation frequency rises, consistent with a relatively fixed neural conduction delay through the auditory brainstem (John & Picton, 2000). Together, amplitude

and phase measures offer a more comprehensive understanding of subcortical encoding of temporal envelope cues than amplitude measures alone.

The synthetic /ba/ syllable developed at Northwestern University by Kraus and colleagues has become one of the most widely used stimuli for assessing speech-evoked FFRs. This 170 ms stimulus contains several acoustically important features, including an onset transient, a voiced stop burst, a formant transition, and a steady-state vowel segment. As a result, it provides a detailed representation of multiple speech cues within a single recording. The FFR evoked by /ba/ typically includes an onset response like auditory brainstem response (ABR) wave V, sustained envelope-following activity representing the fundamental frequency (f0) and low harmonics, and temporal fine-structure components corresponding to formant frequencies.

In adults with NL, the /ba/ stimulus produces clear spectral peaks at the f0 (around 100 Hz) and its harmonics, as well as a representation of the first formant frequency (f1) (approximately 720 Hz). Research demonstrated that accurate neural encoding of f0 in the FFR is associated with better speech-in-noise perception, musical pitch discrimination, and reading skills (Kraus & Nicol, 2005). These findings established the FFR as an important neural marker of auditory abilities relevant to everyday communication. In another study, found that f0 tracking remains relatively robust across different signal-to-noise ratios in listeners with NL, although response quality gradually deteriorates as background noise levels increase (Johnson et al., 2005).

One of the major strengths of the FFR is its reliability. Studies have shown that NL individuals display highly consistent FFR waveforms across recording sessions and even across different laboratories when similar stimuli and recording protocols are used. This consistency has allowed researchers to establish normative databases that can serve as

references when evaluating clinical populations, including individuals with sensorineural hearing loss. Age-related differences in FFR characteristics have also been widely reported. For example, younger adults generally demonstrate larger response amplitudes, shorter latencies, and stronger pitch encoding than older adults; these changes likely reflect age-related declines in subcortical auditory processing that occur independently of peripheral hearing sensitivity (Anderson et al., 2012).

CHAPTER 3

METHODS

Participants

A total of 15 participants with a confirmed diagnosis of auditory neuropathy (AN) and 15 participants with normal hearing (NL) were included in the study. The participant's ages ranged between 16–40 years. Efforts were made to ensure an equal distribution of male and female participants, and selection was based on the following criteria

Participant Selection Criteria for the NL Group

NL sensitivity was confirmed in both ears across octave frequencies ranging from 250 Hz to 8000 Hz for air conduction (AC) thresholds and from 250 Hz to 4000 Hz for bone conduction (BC) thresholds. A pure tone average (PTA) of less than 20 dB HL was considered indicative of NL. In addition, only individuals with speech identification scores greater than 95% were selected for participation along with the presence of transient evoked otoacoustic emissions (TEOAE). Normal middle ear function was verified by A/As-type tympanometry findings and the presence of ipsilateral and contralateral acoustic reflexes. Furthermore, participants showed no evidence of retrocochlear pathology, as confirmed by auditory brainstem response (ABR) testing at slow (11.1/sec) and fast (90.1/sec) repetition rates.

Participant Selection Criteria for the AN Group

Individuals in the age range of 16–40 years who were diagnosed with AN with mild to moderate hearing loss were considered for the study. The diagnosis was established by a qualified audiologist according to the criteria by Starr et al. (1996). Accordingly, all participants exhibited absent ABRs to 90 dB nHL clicks presented at 11.1/s; TEOAEs were robustly present, and absent acoustic reflexes at maximum intensity levels.

Table 3.1

The Demographic Details and Audiological Characteristics of the AN group

Name	Age	Gender	PTA R	PTA L	Tymp R	Tymp L	Reflex	OAE	ABR
S1	27	F	42.5	42.5	As	As	Absent	Present	Absent
S2	19	M	35	45	A	A	Absent	Present	Absent
S3	23	M	42.5	55	A	As	Absent	Present	Absent
S4	21	F	50	43.75	As	A	Absent	Present	Absent
S5	21	F	22.5	22.5	As	A	Absent	Present	Absent
S6	23	M	26.25	48.75	A	A	Absent	Present	Absent
S7	17	F	48.75	49	A	A	Absent	Present	Absent
S8	24	M	1.2	10	As	As	Absent	Present	Absent
S9	39	M	25	30	A	A	Absent	Present	Absent
S10	16	F	50	45	A	A	Absent	Present	Absent
S11	19	F	40	40	A	A	Absent	Present	Absent
S12	29	F	23.75	17.5	A	As	Absent	Present	Absent
S13	31	F	47.5	50	A	A	Absent	Present	Absent
S14	21	F	40	38.75	As	As	Absent	Present	Absent
S15	33	M	49.5	50	A	A	Absent	Present	Absent

Ethical Considerations

Before testing commenced, all participants were informed of the procedures and the study's purpose, and written informed consent was obtained from each participant. All the steps involved in that study adhered to the ethical guidelines for bio-behavioural research involving human subjects of AIISH, Mysuru (AIISH, 2026). The study was approved by the AIISH Institutional Review Board (SH/IRB/M.4/AUD.05/2025-26). Annexure I provide the ethical approval letter.

Test Environment

All tests were carried out in an acoustically and electrically shielded double room with ambient noise within the permissible limits (Frank et al., 1993).

Instrumentation

A calibrated two-channel stand-alone audiometer, AC40 (Interacoustics, Middelfart, Denmark), was used to assess participants' hearing status. AC thresholds, BC thresholds, and speech audiometry were carried out for all participants. Tympanometry and acoustic reflex measurements were performed using a calibrated Titan with an IMP440 Impedance System (Interacoustics, Middelfart, Denmark). Tympanometry was recorded using a 226 Hz probe tone, and acoustic reflex thresholds were measured at 500 Hz, 1 kHz, 2 kHz & 4 kHz in both ipsilateral and contralateral modes for all participants. A calibrated otoacoustic emission analyser, ILO v6 (Otodynamics Ltd., Hatfield, UK), was used to record TEOAE. ABR & frequency following responses (FFR) were recorded using a calibrated four-channel evoked potential system, SmartEP 5.54.21 (Intelligent Hearing Systems, Florida, USA), along with the Opti Amp USB box and multi-channel Opti Amp transmitter 8008. Ultra-shielded ER-2 insert earphones, 10-ohm (Intelligent Hearing Systems, Florida, USA), were used for stimulus presentation. Disposable gold tip trode electrodes (10 mm) and insert earphone foam tips (Etymotic Research, Inc., Illinois, USA) were used for stimulus presentation and recording of ABR and FFR.

Basic Audiological Evaluation

A detailed case history was obtained from all participants to rule out any pathology affecting the auditory system. Otoscopic examination was carried out to exclude impacted wax and other external ear pathologies. Hearing thresholds were assessed for both AC (250 Hz to 8000Hz) and BC (250 Hz to 4000 Hz) following the Modified Hughson-Westlake procedure. Speech identification scores were obtained using the phonetically balanced monosyllabic Word List I (Chandrashekhara, 1972). Tympanometry was performed using a 226 Hz probe tone, and acoustic reflexes were measured at 500 Hz, 1 kHz, 2 kHz, and 4 kHz in both ipsilateral and contralateral modes. TEOAEs were recorded using 260 sweeps of linear click trains presented at 80 dB pe SPL. ABRs were recorded using click stimuli.

Frequency Following Responses

Stimuli

FFRs were recorded for sinusoidal amplitude modulated (SAM) pure tones and the speech syllable /ba/. A 500 Hz pure tone, generated at a 20 kHz sampling frequency, with 100% modulation depth at 16 Hz, 32 Hz, and 64 Hz served as stimuli. The tone was passed through a cosine-squared gating function. The stimuli were generated using the utilities' function available in the IHS software. In addition, FFRs were elicited using a 170 ms synthetic /ba/ syllable. The waveform and spectrum of the /ba/ syllable are shown in Figures 3.1 and 3.2, respectively.

Figure 3.1

Waveform of 170 ms Synthetic /ba/ Stimulus

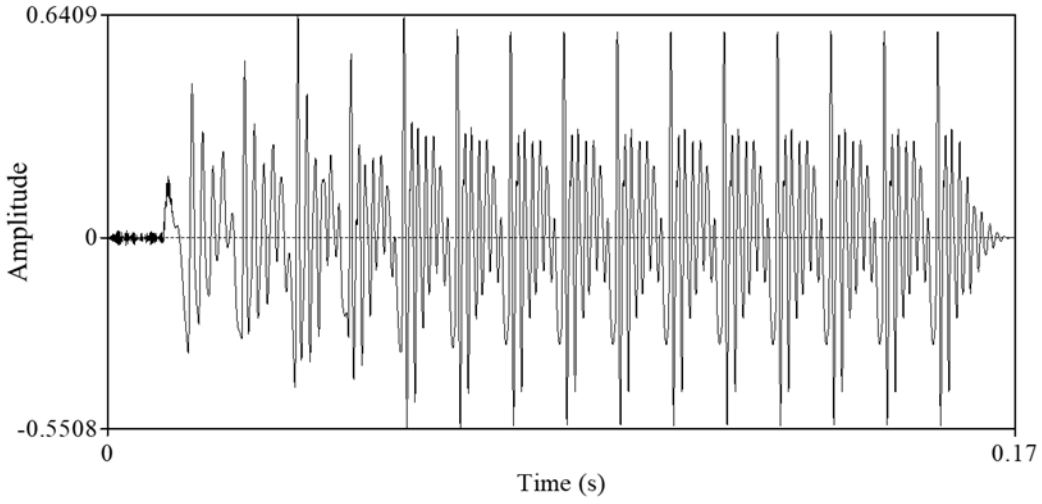
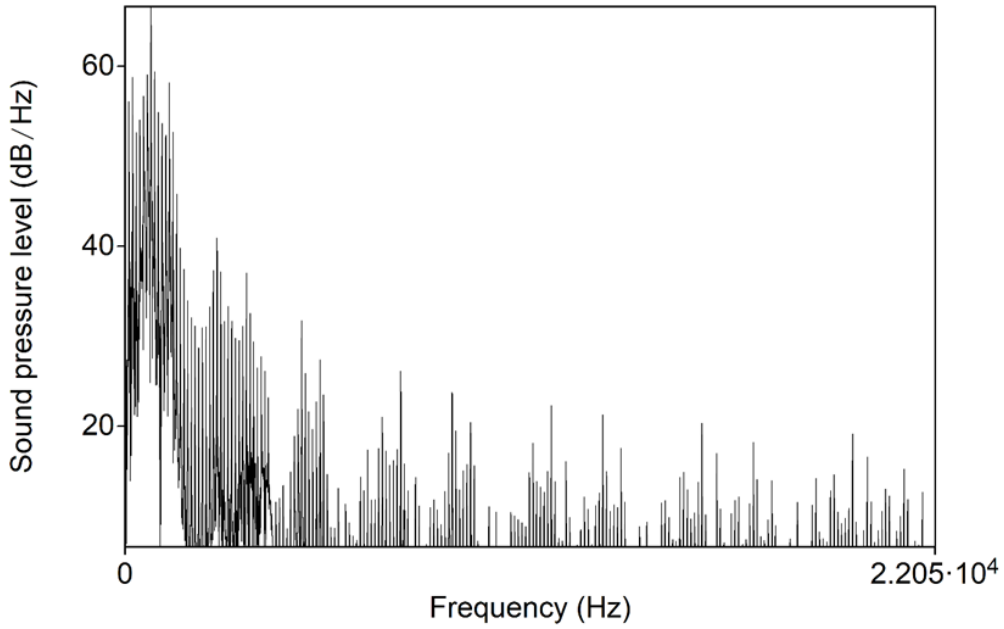


Figure 3.2

Spectrum of 170 ms Synthetic /ba/ Stimulus



Procedure

FFRs were recorded in a sound-treated room using the Advanced Auditory Research Module of SmartEP (IHS). Participants were made to sit in a reclining chair and were instructed as follows, "You will hear sounds in your ear. You do not need to respond to the sounds. Please keep your eyes closed and remain relaxed throughout the testing." The electrode sites were cleaned before placing the disc electrodes (for ABR) or the tip trode (for FFR). The absolute impedance of each electrode was maintained below 5 kΩ, and the inter-electrode impedance was kept below 2 kΩ. Recordings were obtained from the right ear of all participants. FFRs were recorded using a horizontal electrode montage. The non-inverting electrode (gold-foil tip trode) was placed in the right ear canal, the inverting electrode was positioned at the contralateral mastoid, and the ground electrode was placed on the lower forehead. Stimuli were presented to the right ear through shielded ER-2 insert earphones at a repetition rate of 4.1/s and an intensity of 80 dB nHL. Two recordings of 1024 sweeps each

were collected using alternating polarity. The averaged waveform from the two recordings was used for analysis. The responses were amplified 100,000 times with artifact rejection enabled and band-pass filtered between 50 and 3000 Hz for /ba/ and 1 and 3000 Hz for SAM stimuli.

Table 3.2

Acquisition and Stimulus Parameters for Recording FFR

Transducer type	Ultra shielded ER-2 Insert Earphones
Electrodes	• Inverting - Contralateral mastoid (Mi) using cup electrodes • Non inverting - Disposable electrode and insert earphone foam tip: Gold tip trode electrode in the test (Right) ear canal • Ground - lower forehead using cup electrodes
Filter setting	1-3000 Hz for SAM 50 – 3000 Hz for /ba/
Analysis time	200 ms
Gain	100,000
Number of sweeps	1024
Stimulus Rate	4.1/s
Inter-electrode impedance	< 5000 Ω
Intra-electrode impedance	< 2000 Ω
Notch filter	Off

Type of Stimulus	Speech stimuli - /ba/ SAM – Carrier frequency of 500 Hz and 100% modulated at 16 Hz, 32 Hz and 64 Hz
Stimulus Duration	170 ms – /ba/ 150 ms – SAM
Stimulus Polarity	Alternating
Intensity	80dB nHL

Analyses

All FFR recordings, as mentioned above, were obtained using alternating stimulus polarity. The split-buffer averaging function within the IHS SmartEP software was used to store responses as condensation and rarefaction polarities separately. These were later used to obtain two components: Envelope (FFR_{ENV}) - by summing the two polarity FFR, and Temporal Fine Structure (FFR_{FS}) - by subtracting one polarity FFR from the other (Aiken & Picton, 2008; Krishnan, 2002).

Spectral analysis was performed using the Fast Fourier Transform (FFT) in a custom matrix laboratory (MATLAB) script. Offline filtering was performed in MATLAB before obtaining the FFT to analyse the spectral amplitudes. The FFR_{ENV} were analysed to extract the spectral amplitude at the modulation frequency for SAM tones and fundamental frequency for syllable /ba/. The FFR_{FS} of /ba/ stimulus was analysed to extract spectral amplitudes at frequencies corresponding to the first and second formant frequencies (700 Hz and 1200 Hz respectively). The extracted data were tabulated using the statistical package for the social sciences (SPSS) version 26 and subjected to appropriate statistical analyses.

CHAPTER 4

RESULTS

The current study examined frequency-following responses (FFR) in individuals with normal hearing (NL) and auditory neuropathy (AN). Prior to the commencement of the experiments, the presence of true neural responses was ensured using two control procedures. First, the probe tube was clamped during recording. Clamping the tube prevents the stimulus from being heard; therefore, no responses should be obtained if the recorded activity is neural. Conversely, if responses persist despite clamping, they are likely attributable to stimulus artifact rather than neural activity. Second, recordings were obtained from a participant with profound hearing loss to ensure that no neural response was present under these conditions.

Figure 4.1 illustrates the responses recorded for the */ba/* stimulus, and Figure 4.2 illustrates the responses recorded for sinusoidal amplitude modulated (SAM) at 64 Hz stimulus from a NL participant (with and without probe tube clamping) and from a participant with profound hearing loss. As shown in Figure 4.1 and Figure 4.2, no responses were recorded when the tube was clamped or in the individual with profound hearing loss. Together, these procedures and the usage of electrically shielded earphones confirmed that the responses recorded in the main experiment were physiological and not contaminated by stimulus artifacts. After ruling out contamination by stimulus artifacts, the subsequent experiments were conducted.

Figure 4.1

FFR Recordings for the /ba/ Stimulus under Unclamped and Clamped Probe Tube Conditions in a NL Participant and in a Participant with Profound Hearing Loss

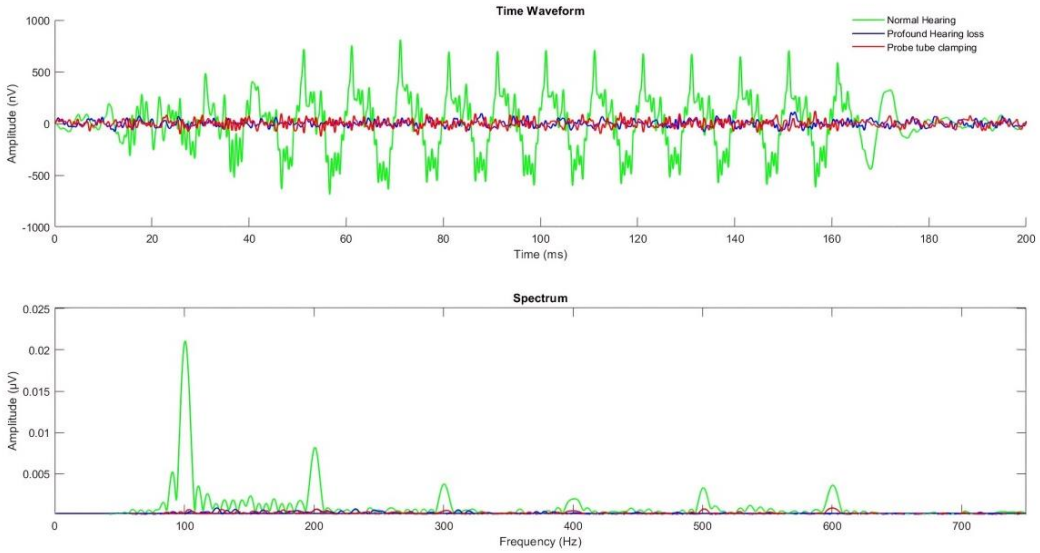
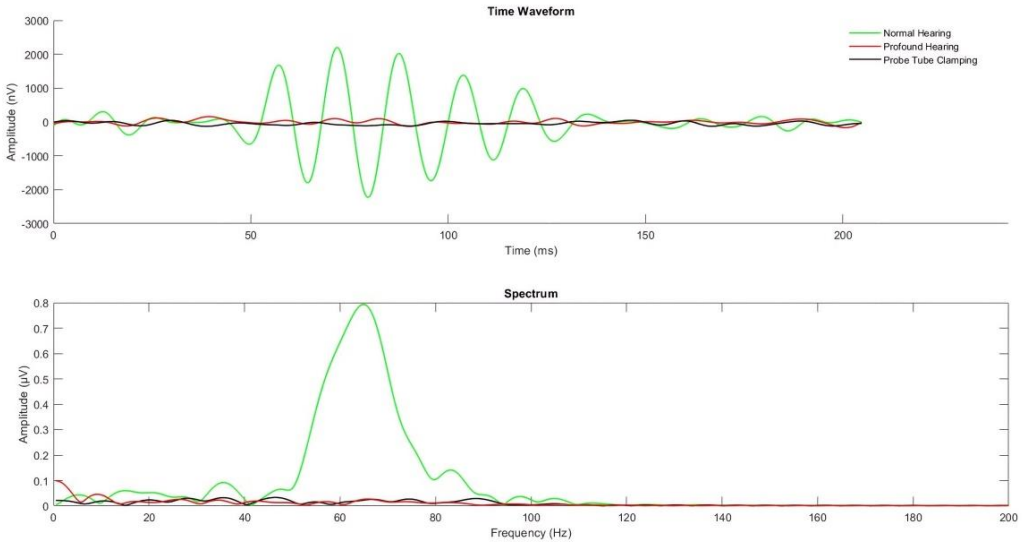


Figure 4.2

FFR Recordings for the SAM 64 Hz Stimulus under Unclamped and Clamped Probe Tube Conditions in a NL Participant and in a Participant with Profound Hearing Loss



Envelope Following Responses (FFR_{ENV}) for Amplitude-Modulated Signals in Individuals with NL and AN

Results in this section address the objectives 1 and 2. Figure 4.3, Figure 4.4 & Figure 4.5 shows the grand - averaged FFR_{ENV} waveforms and spectrum across three modulation frequencies in the NL and AN group. Figure 4.6 shows the median, quartile deviations, and individual data points for FFR_{ENV} amplitude across three modulation frequencies in the NL and AN groups. As shown in Figure 4.3, Figure 4.4 & Figure 4.5, the amplitude of FFR_{ENV} was higher in individuals with NL than in those with AN. In addition, the amplitude of FFR_{ENV} decreased with increasing modulation frequency.

Figure 4.3

Waveform and Spectrum FFR_{ENV} in Response to the SAM 16 Hz Stimulus in the NL (black) and AN (red)

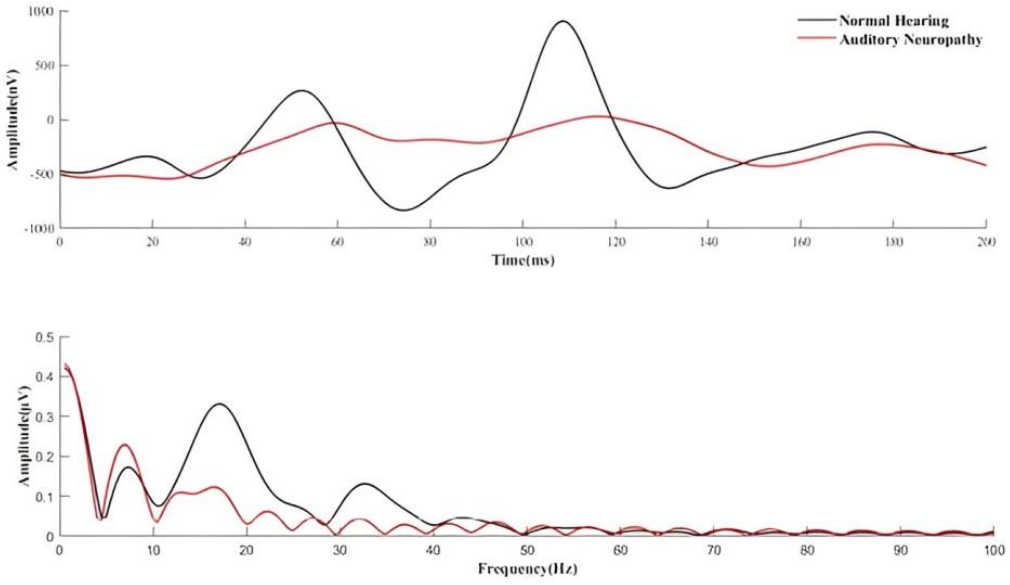


Figure 4.4

Waveform and Spectrum FFR_{ENV} in Response to the SAM 32 Hz Stimulus in the NL (black) and AN (red)

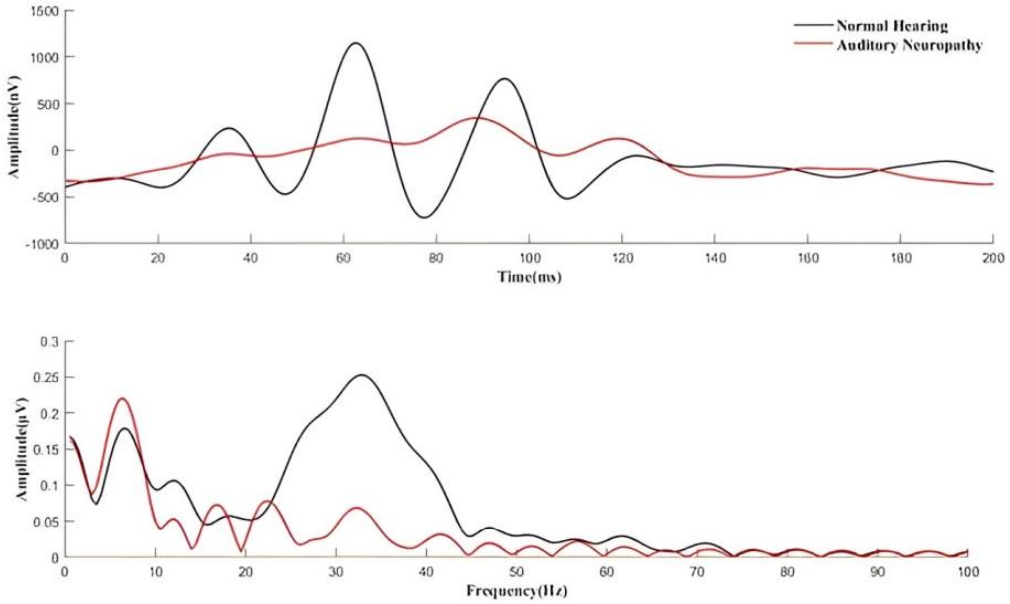


Figure 4.5

Waveform and Spectrum FFR_{ENV} in Response to the SAM 64 Hz Stimulus in the NL (black) and AN (red)

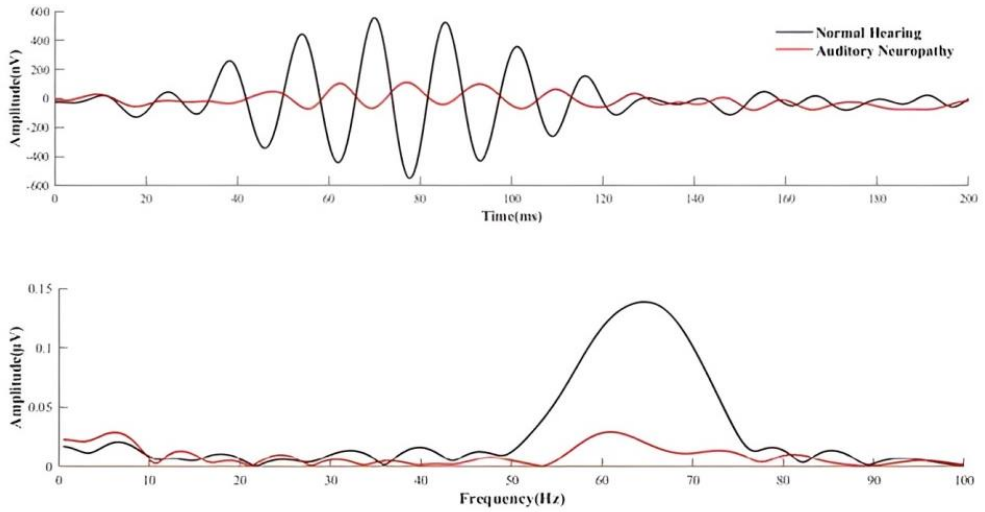
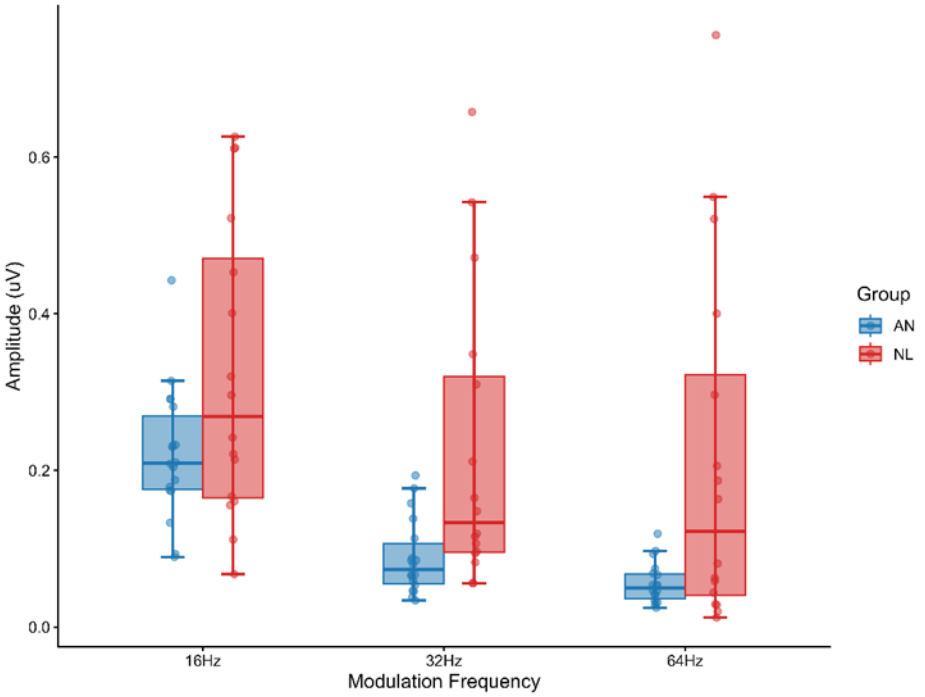


Figure 4.6

Median, Quartile Deviation, and Individual Data Points of FFR_{ENV} across the Two Groups at Three Different Modulation Frequencies



Generalized linear mixed model (GLMM) was fitted for FFR_{ENV} amplitudes. Group and modulation frequencies were included as fixed effects, and participant-specific intercepts were included as random effects. Models were estimated using the maximum likelihood method with a gamma distribution and a log link function. The Kolmogorov–Smirnov test indicated that the data followed a gamma distribution, and the Diagnostics for Hierarchical Regression models (DHARMa) dispersion test was not significant, indicating that the residuals were neither over dispersed nor under dispersed. Table 4.1 presents the Type II Wald chi-square tests, random effects (intercepts), and the results of the dispersion tests FFR_{ENV} amplitudes.

Table 4.1

Statistical Analyses of FFR_{ENV} across Two Groups and Three Modulation Frequencies

Groups	$\chi^2(1) = 21.1$; $p < .01$
Modulation frequency	$\chi^2(2) = 49.8$; $p < .01$
Groups x Modulation frequency	$\chi^2(2) = 7.9$; $p < .01$
Dispersion test	D=0.06, p = 0.79
Random effects	Est = 1.5
	Z = 9.3
	$p < .01$

Results indicated a significant main effect of group and modulation frequency on FFR_{ENV} amplitudes. The interaction between group and modulation frequency was also significant. Based on the GLMM results, post hoc comparisons were conducted using estimated marginal means with Bonferroni correction (Table 4.2).

Post hoc pairwise comparisons revealed the following:

- Within the AN group:
    - The amplitude of FFR_{ENV} was significantly higher at 16 Hz than at 32 Hz and 64 Hz.
    - No significant difference was observed between 32 Hz and 64 Hz.
- Within the NL group:
    - The amplitude at 16 Hz was significantly higher than at 64 Hz.
    - No significant differences were observed between 16 Hz and 32 Hz or between 32 Hz and 64 Hz.
- Between-group comparisons:

- The amplitude of FFR_{ENV} in the NL group was significantly higher than in the AN group at 32 Hz and 64 Hz.
- At 16 Hz, the amplitudes were comparable between the two groups.

Table 4.2

Results of Post Hoc Comparisons of Estimated Marginal Means with Bonferroni Correction across Different Modulation Frequencies

Group	Modulation frequencies	Z ratio	p value
Auditory Neuropathy	16 Hz – 32 Hz	4.7	< .001
	16 Hz – 64 Hz	6.9	< .001
	32 Hz – 64 Hz	2.1	.08
Normal Hearing	16 Hz – 32 Hz	1.9	.16
	16 Hz – 64 Hz	2.5	.03
	32 Hz – 64 Hz	0.6	1.0

Table 4.3

Results of Post Hoc Comparisons of Estimated Marginal Means with Bonferroni Correction Between the Two Groups

Modulation frequency	Z ratio	p value
16 Hz	1.44	0.144
32 Hz	3.69	0.0002
64 Hz	4.6	0.0001

Envelope Following Responses (FFR_{ENV}) for Speech Stimulus /ba/ in Individuals with NL and AN

The Shapiro–Wilk test indicated that the FFR amplitudes were not normally distributed. Therefore, nonparametric tests were used for the comparisons. A Mann–Whitney U test was performed to compare the /ba/ FFR_{ENV} amplitudes between the NL and AN groups. The analysis revealed a statistically significant difference in amplitudes between the two groups, with the AN group showing lower amplitudes than the NL group [$U = 58$, $Z = 2.79$, $p < .001$]. Figure 4.7 shows the grand averaged FFR_{ENV} waveform and spectrum of /ba/ in the AN and NL groups. Figure 4.8 shows the median, quartile deviation, and individual data points of FFR_{ENV} for the /ba/ stimulus.

Figure 4.7

Grand Average Waveform and Spectrum FFR_{ENV} in Response to the /ba/ Stimulus in the NL (black) and AN (red)

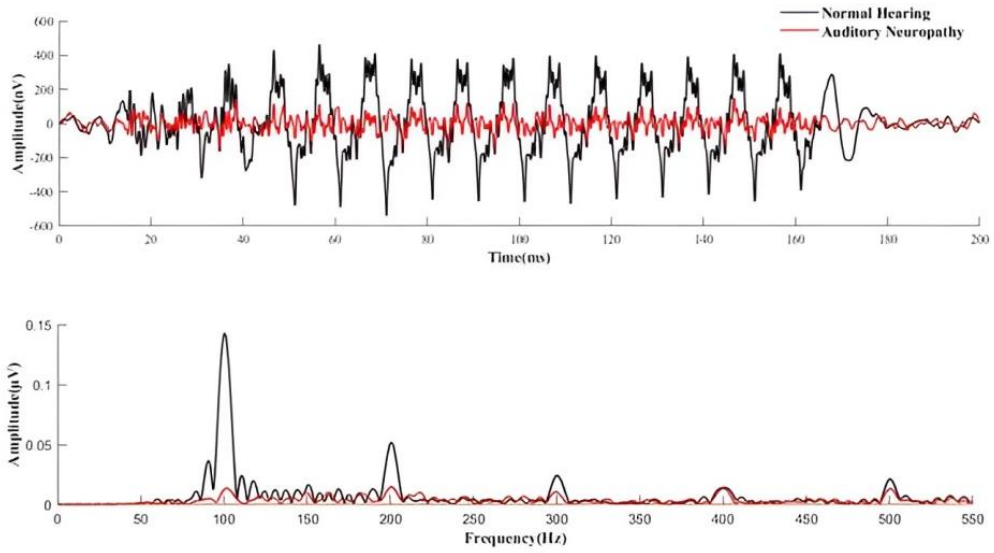
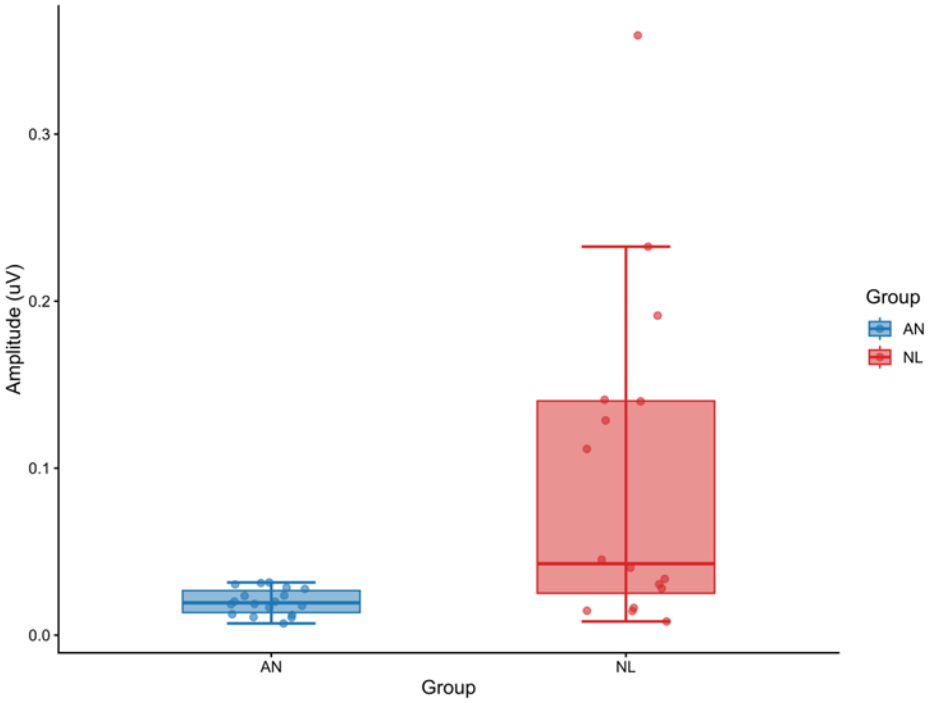


Figure 4.8

Median, Quartile Deviation, and Individual Data Points of FFR_{ENV} across the Two Groups for the /ba/ Stimulus



Fine structure

The Figure 4.10 shows the median, quartile deviation, and individual data points of FFR_{FS} amplitudes in the NL and AN groups for the first and second formant frequencies. Figure 4.9 shows the FFR_{FS} waveforms and spectra of the grand-averaged FFR_{FS} responses. The figure demonstrates that the NL group exhibited clear peaks at the first and second formant frequencies, whereas the AN group showed reduced amplitudes for these peaks.

GLMM were fitted for FFR_{FS} amplitudes. Group and formant frequencies were included as fixed effects, and participant-specific intercepts were included as random effects. Models were estimated using the maximum likelihood method with a gamma distribution and a log link function. The Kolmogorov–Smirnov test indicated that the data followed a gamma distribution, and the Diagnostics for Hierarchical Regression models (DHARMa) dispersion

test was not significant, indicating that the residuals were neither overdispersed nor underdispersed. Table 4.4 presents the Type II Wald chi-square tests, random effects (intercepts), and the results of the dispersion tests FFR_{FS} amplitudes.

Table 4.4

Statistical Analyses of FFR_{FS} across Two Groups and Two Formant Frequencies

Groups	$\chi^2(1) = 2.61$; $p > .001$
Formant frequency	$\chi^2(1) = 56.09$; $p < .001$
Groups x formant frequency	$\chi^2(1) = 0.49$; $p > .001$
Dispersion test	D=0.11, p = 0.37
Random effects	Est= 4.4
	Z = 17.1
	p < .001

Statistical analysis revealed no significant main effect of group on FFR_{FS} amplitudes, whereas a significant main effect of formant frequency was observed. The interaction between the group and formant frequency was found to be non-significant as presented in the Table 4.4.

Figure 4.9

Grand Average Waveform and Spectrum FFR_{FS} in Response to the /ba/ Stimulus in the NL (black) and AN (red)

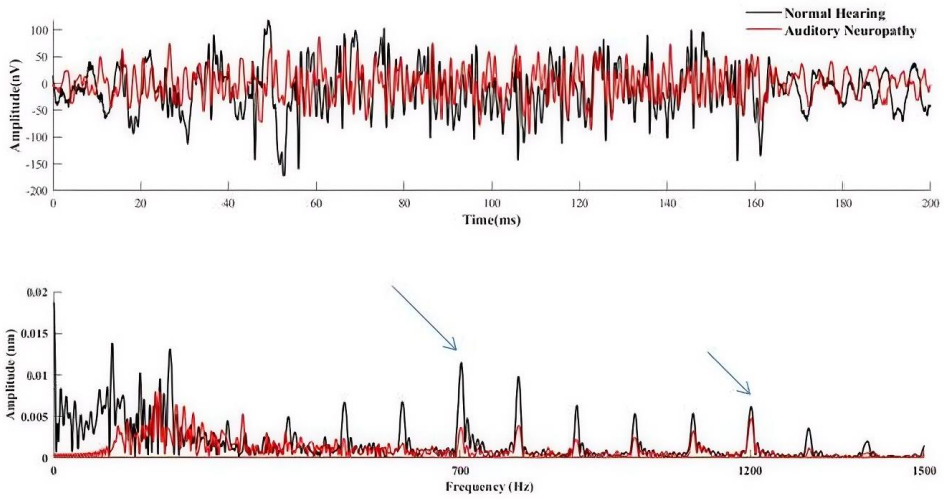
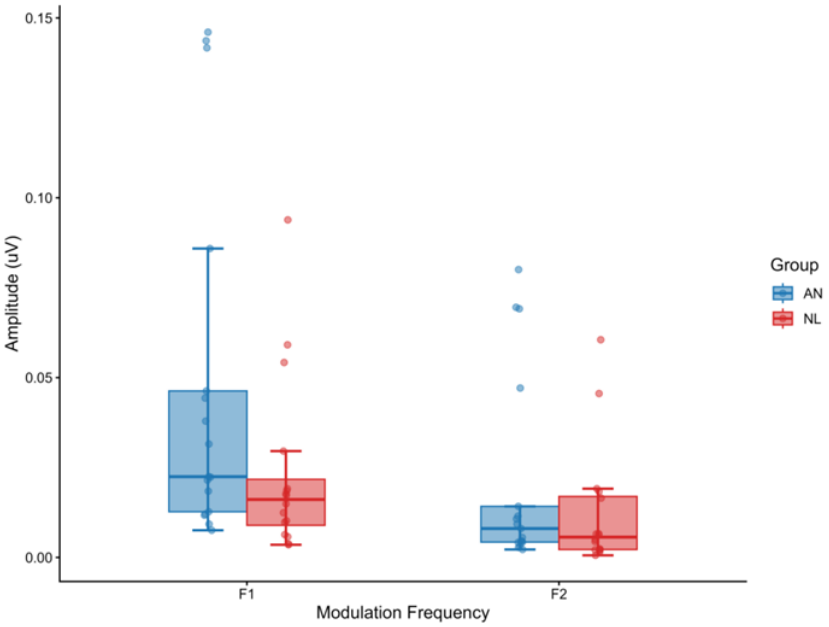


Figure 4.10

Median, quartile deviation, and individual data points of FFR_{FS} across the two groups for the /ba/ stimulus.



Chapter 5

Discussion

The current study investigated the frequency following response (FFR) in individuals with auditory neuropathy (AN) and those with normal hearing (NL). The results are discussed in light of current understanding of the disorder and its effects on subcortical auditory function.

5.1 Envelope Following Response (FFR_{ENV}) to Sinusoidal Amplitude Modulated (SAM) Tones

The first objective of the present study was to compare the spectral amplitude of FFR in response to a 500 Hz SAM pure tone at 16 Hz, 32 Hz and 64 Hz between individuals with AN and those with NL. The results revealed a significant main effect of group and modulation frequency on FFR_{ENV} amplitudes. The interaction between group and modulation frequency was also significant. Post hoc analyses revealed that, in both groups, FFR_{ENV} amplitude reduced with increasing modulation frequency. The AN group showed significantly lower amplitudes than the NL group at higher modulation frequencies. However, at the low modulation frequency of 16 Hz, the FFR_{ENV} amplitudes were comparable between the two groups.

These results are in line with expectations. Several behavioural studies have shown that the sensitivity of the auditory system to modulation frequencies reduces as the modulation frequency increases (JORIS et al., 2004; Wang et al., 2012; Whiteford & Oxenham, 2023). Furthermore, higher modulation frequencies are typically processed at lower auditory centres such as the brainstem, whereas lower modulation frequencies are processed at cortical regions (Farahani et al., 2021; Gransier et al., 2021; Overath et al., 2012). It is well known that the amplitudes of potentials generated by cortical regions are typically

higher than those generated by the brainstem (Kohl et al., 2022; Picton et al., 1974). These two factors, namely (a) reduced sensitivity of the auditory system with increasing modulation frequency and (b) the involvement of cortical regions in processing lower modulation frequencies, may be responsible for the reduction in FFR_{ENV} amplitude with increasing modulation frequency.

Between-group comparisons revealed that at 16 Hz, FFR amplitudes were comparable between the two groups, whereas at 32 Hz and 64 Hz, the AN group had significantly lower FFR amplitudes than the NL group. These findings are completely in line with the current understanding that a disturbance of neuronal synchronization in the auditory nerve and its afferent pathways is the main pathophysiological mechanism in AN (Rance & Starr, 2015; Starr et al., 1996). The integrity of temporal coding in these pathways is crucial for the FFR, which represents phase-locked neuronal activity from subcortical generators such as the inferior colliculus and cochlear nucleus. Phase-locked subcortical responses are expected to be diminished or non-existent when the synchronization of auditory nerve discharges is disrupted, which is the characteristic of AN. The current study's significantly lower FFR_{ENV} amplitudes, specifically at 32 Hz and 64 Hz modulation frequencies in the AN group is due to reduced neuronal synchronization.

The finding that the AN group's FFR_{ENV} amplitude was significantly lower at higher modulation frequencies (32 Hz and 64 Hz) but not at 16 Hz is in line with earlier modulation detection studies. Individuals with AN have poorer amplitude modulation (AM) detection thresholds, especially at higher modulation rates, which is indicative of decreased neuronal synchronization in processing abrupt temporal changes (Yuvaraj & Jayaram, 2016). Similarly, those with AN had a disproportionate weakness in processing quicker temporal envelope cues, requiring much deeper modulation depths to detect both amplitude and frequency modulation (Narne, 2013). The pattern observed in the current study, wherein 16 Hz responses were

relatively preserved while 32 Hz and 64 Hz responses were significantly reduced, suggests that individuals with AN may retain some residual capacity for encoding slower envelope fluctuations but are considerably impaired in tracking the faster temporal modulations that are critical for speech understanding. From a neural coding perspective, these findings are coherent with what is known about the generators of the FFR_{ENV}. Neurons in the inferior colliculus phase-lock effectively to the modulation envelopes of AM tones, and this phase-locking capacity diminishes at higher modulation rates, reflecting the upper limit of neural synchrony within the ascending auditory pathway (Batra et al., 1989). In AN, the disruption of synchrony at the level of the inner hair cell-auditory nerve synapse or the auditory nerve itself would further reduce the effective upper limit of modulation encoding, explaining the steeper amplitude reduction seen in the AN group compared to the NL group at higher modulation frequencies. Furthermore, as mentioned earlier, lower modulation frequencies are processed in cortical centres, and there is evidence showing that cortical evoked responses are preserved in individuals with AN (V. K. Narne, 2013; V. kumar Narne & Vanaja, 2008).

5.3 FFR_{ENV} in Response to the /ba/ Speech Stimulus

The third objective was to compare the spectral amplitude of the FFR_{ENV} elicited by the /ba/ syllable between individuals with AN and those with NL. Results revealed a statistically significant difference, with the AN group demonstrating significantly lower FFR_{ENV} /ba/ amplitudes than the NL group. This finding is in line with the hypothesis that impaired temporal coding at the auditory nerve level in AN would manifest as reduced neural fidelity in encoding the envelope of complex speech signals.

The synthetic /ba/ stimulus used in the current study is a 170 ms complex stimulus containing an onset transient, a formant transition, and a steady-state vowel segment. The FFR_{ENV} component of the speech-evoked FFR represents the neural encoding of the fundamental frequency (f0) and the temporal envelope of the vowel portion of the stimulus.

Kraus and Nicol, (2005) demonstrated that accurate neural encoding of f0, as indexed by the FFR, is associated with better speech-in-noise perception, and f0 tracking is relatively robust in NL group (Johnson et al., 2005). The significantly attenuated FFR_{ENV} /ba/ amplitudes in the AN group in the current study suggest that individuals with AN are unable to faithfully encode speech f0, which would directly impair their ability to utilise voicing cues in everyday communication, particularly in noisy environments.

5.4 Fine Structure Following Responses (FFR_{FS}) to the /ba/ Stimulus.

In addition to the FFR_{ENV}, the present study also examined the FFR_{FS} evoked by the /ba/ stimulus, specifically at the first (F1) and second (F2) formant frequencies. Statistical analysis revealed no significant main effect of group on FFR_{FS} amplitudes, suggesting that the fine structure responses at F1 and F2 were not significantly different between the NL and AN groups. However, a significant main effect of formant frequency was observed, with amplitude decreasing progressively with increasing formant frequency, i.e., the F2 response was smaller than the F1 response in both groups.

First, the statistical non-significance of group effect on FFR_{FS} may be partly attributable to the limited sample size of 15 participants per group and the associated variability in FFR_{FS} responses, which are inherently smaller in amplitude than envelope-following responses. Second, individual variability in the site and extent of auditory nerve pathology in the AN group may have resulted in a heterogeneous pattern of fine structure responses across participants, obscuring a statistically significant group effect. In AN, the site of lesion can vary from presynaptic inner hair cell dysfunction to postsynaptic auditory nerve demyelination or ganglion cell loss (Rance & Starr, 2015), and it is possible that individuals with predominantly presynaptic lesions may retain relatively better fine structure encoding compared to those with more distal axonal pathology.

The significant main effect of formant frequency, with higher formant frequencies eliciting smaller FFR_{FS} amplitudes in both groups, is consistent with the physiology of phase-locking in the auditory brainstem. Neural phase-locking capacity decreases substantially above 1 kHz, and since F2 of /ba/ is approximately at 1.2 kHz, lower FFR_{FS} amplitudes at F2 are anticipated (Bidelman & Powers, 2018). This pattern has been reported in prior FFR studies and reflects a fundamental property of subcortical auditory processing rather than pathology-specific change.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Summary

The present study investigated frequency following responses (FFR) in individuals with auditory neuropathy (AN) and normal hearing (NL) to examine neural encoding of temporal envelope and fine-structure cues. A total of 30 participants (15 AN, 15 NL) aged between 16–40 years were included. FFRs were recorded in response to sinusoidal amplitude-modulated (SAM) tones at three modulation frequencies (16 Hz, 32 Hz, and 64 Hz) and a synthetic /ba/ syllable. Spectral amplitudes of the envelope following response (FFR_{ENV}) and fine structure following response (FFR_{FS}) were extracted and compared between the two groups using appropriate statistical methods.

Results showed that FFR_{ENV} amplitudes were significantly lower in the AN group compared to the NL group at 32 Hz and 64 Hz modulation frequencies, while amplitudes at 16 Hz were comparable between the groups. Within both groups, FFR_{ENV} amplitude decreased progressively with increasing modulation frequency. For the /ba/ syllable, the AN group demonstrated significantly lower FFR_{ENV} amplitudes than the NL group, indicating impaired neural encoding of the fundamental frequency (f0) of speech. In contrast, FFR_{FS} amplitudes for the /ba/ stimulus showed no significant difference between the groups, though a significant effect of formant frequency was observed, with F2 amplitudes being smaller than F1 in both groups.

Conclusions

The findings of the present study lead to the following conclusions:

- **FFR_{ENV} to SAM tones is significantly reduced in AN** at higher modulation frequencies (32 Hz and 64 Hz), reflecting impaired subcortical temporal envelope encoding due to disrupted auditory nerve synchrony.
- **At 16 Hz, FFR_{ENV} amplitudes are comparable between AN and NL groups**, suggesting that slower envelope fluctuations which have a cortical contribution may be relatively preserved in individuals with AN.
- **FFR_{ENV} amplitude decreases with increasing modulation frequency** in both groups, consistent with the known limits of neural phase-locking in the ascending auditory pathway.
- **FFR_{ENV} to the /ba/ syllable is significantly attenuated in the AN group**, indicating impaired neural encoding of speech f0, which likely underlies the disproportionately poor speech perception abilities observed in this population.
- **FFR_{FS} to the /ba/ syllable did not differ significantly between groups**, possibly due to the limited sample size, heterogeneity of lesion sites in AN, and the physiological limitations of phase-locking at higher formant frequencies.
- **FFR holds as a promising objective clinical tool** for quantifying the degree of temporal coding deficit in individuals with AN and may assist in guiding rehabilitation decisions.

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Annexure I

All India Institute of Speech and Hearing
(An autonomous Institute under the
Ministry of Health and Family Welfare, Govt. of India)
Center of Excellence - Assessed & Accredited by NAAC with 'A' Grade
ISO 9001:2015 Implemented Institute
Manasagangothri, Mysuru-570006

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INSTITUTE REVIEW BOARD (IRB) APPROVAL FOR BIO-BEHAVIOURAL RESEARCH PROPOSAL INVOLVING HUMAN SUBJECTS AT AIISH

AIISH Institute Review Board (IRB)

Ref: SH/IRB/M.4/AUD.05/2025-26 **Date: 06/01/2026**

Title of the Dissertation	: Frequency Following Response in Individuals with Auditory Neuropathy Spectrum disorder
Name of the Investigator	: Ms. Aishwarya Nayak
Guide	: Dr. Ajith Kumar U
Co-Guide (if any)	: Nil
Proposed Duration of the Project	: 08 months
Source of funding	: Non-funded Master's dissertation
Estimated budget	: Nil
Reference Number of the Project	: Nil
Date on which the IRB meeting was held	: 02.01.2026
A clear statement of the decision reached IRB meeting (In the vent of the proposal is not approved, a statement of reasons for the same must be indicated)	**: APPROVED**
Advice & suggestions (if any)	: NIL

Signature & Name of the Member Secretary-IRB
Dr. Animesh Barman
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