Longitudinal Comparison of Auditory Steady-State Evoked Potentials in Preterm and Term Infants: The Maturation Process

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Abstract

Introduction Preterm neonates are at risk of changes in their auditory system development, which explains the need for auditory monitoring of this population. The Auditory Steady-State Response (ASSR) is an objective method that allows obtaining the electrophysiological thresholds with greater applicability in neonatal and pediatric population.

Objective The purpose of this study is to compare the ASSR thresholds in preterm and term infants evaluated during two stages.

Method The study included 63 normal hearing neonates: 33 preterm and 30 term. They underwent assessment of ASSR in both ears simultaneously through insert phones in the frequencies of 500 to 4000Hz with the amplitude modulated from 77 to 103Hz. We presented the intensity at a decreasing level to detect the minimum level of responses. At 18 months, 26 of 33 preterm infants returned for the new assessment for ASSR and were compared with 30 full-term infants. We compared between groups according to gestational age.

Results Electrophysiological thresholds were higher in preterm than in full-term neonates ($p < 0.05$) at the first testing. There were no significant differences between ears and gender. At 18 months, there was no difference between groups ($p > 0.05$) in all the variables described.

Conclusion In the first evaluation preterm had higher thresholds in ASSR. There was no difference at 18 months of age, showing the auditory maturation of preterm infants throughout their development.

Keywords
- evoked potentials
- auditory
- newborn
- infant
- premature
- electrophysiology

Introduction

The maturational development of the auditory system occurs in the peripheral and central auditory systems. The cochlear ability to capture stimuli is functional around the 25th week of intrauterine life, but remains in constant development until birth. The central auditory system is immature at birth. The period of greatest neuronal maturation occurs until the first two years of life, leading the brainstem maturation. Nevertheless, the thalamic-cortical portion remains in

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continuous development during childhood and adolescence up to 15 years old.[1–5]

Preterm neonates are considered at risk for changes in the development of the auditory system. Thus, there may be delays related to the maturational development by neurological immaturity due to interrupting the process of anatomical cortex forming, as well as clinical complications in the neonatal period. Therefore, auditory monitoring of this population is necessary for their general development.[2,6–8]

Thus, the first years of life are critical for child development, including the acquisition and development of language, since it is intrinsically related to the auditory nervous system maturation.[9–11] The pediatric hearing assessment of children must be carried out differently from adults. In this way, the audiologist has to give more attention to each phase of the child maturational and development.

Since the advancement of Newborn Hearing Screening (NHS), the audiological diagnosis has been early performed.[7] The literature indicates that the electroacoustic methods added to the electrophysiological and behavioral methods[12–14] provide a more accurate diagnosis, identifying the configuration, type, and degree of hearing loss. It is essential for appropriate intervention and child development.[6,15,16] The Auditory Steady-State Evoked Potential (ASSR) is included in electrophysiological methods.[15]

The ASSR has been extensively studied in recent years, detecting objectively the electrophysiological thresholds, which are close to the auditory behavioral thresholds.[9,12,17,18] Thus, the ASSR has a greater applicability in neonatal and pediatric populations, whereas this age group may not have cognitive and/or motor conditions for reliable behavioral responses.[15,18] Through the ASSR, the audiologist can evaluate four specific frequencies in both ears simultaneously.[9,19] This is possible due to continuous stimulation, which are modulated in amplitude and/or frequency.[20,21] In addition, the responses can be measured during natural sleep, facilitating the clinical applicability for ASSR.[22]

Some researchers have suggested the audiological monitoring through Auditory Brainstem Response (ABR),[4,23–25] especially in children aged less than six months, which cannot respond to the behavioral assessment. The ABR is the main electrophysiological method to identify changes in neural synchrony in preterm neonates. However, the ASSR can also be useful for this population, identifying the neurological maturation for different intensities of acoustic stimuli.

Based on these points, this study aimed to compare the findings of ASSR between preterm and term tested in two stages, and the possible association with the ear and gender in each group.

Methods

This research is characterized as a cohort, comparative and contemporary study performed in two data collection stages. The Scientific Committee and Research Ethics Committee evaluated and approved the project (protocol n°. 11–137 and 2.011.039). Still emphasizing the completeness of Resolution 466/12 which deals with human research, only the neonates whose parents or guardians signed the Informed Consent participated of this study.

This study included all newborns with no risk factors for hearing loss,[26] with otoacoustic emissions present and no middle ear disorders. These procedures are suggestive of normal hearing up to the outer hair cells. Our research excluded neonates who presented syndromes associated with hearing loss, with the presence of cranio-facial malformations, family history of sensorineural hearing loss, neurological disorders, infections or congenital abnormalities, bacterial meningitis, hyperbilirubinemia level of exsanguination transfusion and Apgar 0–4 at 1 minute or 0–6 at 5 minutes.

We considered the neonates preterm when the gestational age was less than 37 weeks, according to the classification of the World Health Organization.[27]

The sample consisted of 63 neonates, which we divided into two groups: the study group, consisting of 33 preterm neonates, and the control group, consisting of 30 term neonates. We evaluated all neonates with transient evoked otoacoustic emissions (TEOAE), followed by medical evaluation, acoustic impedance measurements with probe of 1000Hz, and ASSR assessment.

The TEOAE were measured with the equipment model Scout, brand Biologic. The criterion of normality was considered when the signal/noise ratio (S/R) was greater or equal than 6 dB in three consecutive frequencies, with reproducibility of 75% in each frequency and overall reproducibility greater or equal to 70%, as suggested by some researchers.[13]

An otorhinolaryngologist carried out the evaluation of the external and middle ear conditions. The acoustic impedance measurements were done using AT235H, Interacoustics brand equipment with 1000 Hz probe, based on protocols found in recent literature.[14,28] All neonates had tympanometric curve type 'A', according to Jerger.[29] This curve is visible when the peak of maximum compliance is between +100 and -100 daPa and the volume of the middle ear between 0.3 and 1.6 ml.

We conducted the ASSR with Smart EP equipment with two channels, Intelligent Hearing Systems (IHS) brand (IHS, Miami, FL), with neonates in natural sleep. We presented each multiple simultaneous stimulus bilaterally through ER-3A insert earphones and obtained the capture of responses by surface electrodes.

The reference electrodes were placed on the right (M2) and left (M1) mastoid, and the active (Fz) and the ground (Fpz) on the forehead. To reduce electrical impedance between the skin and the electrode, we cleaned the skin with gauze and Nuprep. We maintained impedance at or below 3 Kohms.

We determined the lowest level of response using the descendent method. We made a complex acoustic signal, consisting of carrier frequencies of 500, 1000, 2000, and 4000 Hz. We modulated the stimuli were modulated with amplitudes of 77, 85, 93, and 101 Hz in the left ear, and 79, 87, 95, and 103 Hz in the right ear.[6,13,16,22,30,31]

The initial intensity of the stimulus was 60 dB HL to a minimum of zero dB HL. The decrease in intensity was made of 20 dB HL steps and increase of 10 dB HL steps. We used a variation of 5 dB steps to determine the electrophysiological thresholds.[10] The research of the minimum levels of response during the testing of the ASSR was made in dB SPL, but we converted the results to dBNA. According to the conversion table of the equipment used, the value of this conversion is below 26dB for the frequency of 500Hz, 11dB for 1000Hz, 13dB for 2000Hz, and 19dB for 4000Hz.[13]

The software in the IHS equipment automatically calculated the presence of the response, considering the amplitude and phase analysis of the spectral components generated by the multi-frequencial stimuli and amplitude modulated (signal amplitude > 0.0125 microvolts amplitude and noise < 0.05 microvolts). The frequency peaks corresponding to the modulation frequency were considered valid when statistically higher than the noise level. For this, the software used the statistical test F, already installed in the equipment, which considered the response present when the signal and noise ratio was higher or equal ($\geq$) to 6.13 dB in the corresponding frequency and by 5Hz on each side.

The responses obtained from the software were analyzed by two examiners that controlled the responses of the vector and noise, as well as the likelihood values obtained during the whole examination.

The study was conducted in two stages, comparing both groups. The first evaluation took place between the 6th and 15th day of life in term neonates and between the 20th and 28th day of life in preterm neonates. After this, they were invited to perform the second stage at 18 months of age. This difference between the dates of assessment at first testing ensured the correction of gestational age in preterm infants.[4,6,23]

At 18 months old, of the 33 preterm selected for this study, 26 returned, being reevaluated through the ASSR. We made comparisons according to the corrected gestational age.

We generated the database on the Excel program and analyzed it with SPSS (Statistical Package for Social Sciences) software, version 20.0. Continuous variables are described as mean, standard deviation, minimum, and maximum and the categorical variables are presented by absolute and relative frequencies. To compare continuous variables between groups we used the Student *t*-test for independent samples. In comparisons of categorical variables between groups, we applied the Chi-square Pearson test. To compare the right and left ears, we used the Student *t*-test for paired samples. The level of statistical significance was 5% ($p \leq 0.05$).

Results

In the study group, the gestational age ranged from 32 to 36 weeks, in which 15 neonates were girls and 18 boys. For the control group, the gestational age ranged from 37 to 40 weeks, in which 15 infants were girls and 15 boys. Descriptive data are described in ►**Table 1**.

The results from this research showed that there was a statistically significant difference ($p \leq 0.05$) between the groups at the first testing. This difference was found for the four frequencies analyzed through the ASSR. The minimum levels of response were higher in preterm than in full-term neonates. These differences were not found at the second testing. The average of the minimum level of ASSR reduced in 9.32 dB in preterm group (►**Tables 2** and **3**). There was no statistically significant difference for the ear and gender in both groups ($p > 0.05$) (►**Tables 2**, **4**, and **5**).

In addition, the threshold of 500Hz was higher than the other frequencies.

Discussion

Other researchers assert that hearing maturation is an influential factor in the electrophysiological responses for the auditory evoked potentials (AEP)[4,7,12,31–33] in neonatal and pediatric population. So, the thresholds decrease with the advancing age. Electrophysiological assessment with ABR and ASSR show lower thresholds in adults those in neonates, demonstrating the maturational process.[4,23,34–36]

In this study, a comparison between groups at the first testing showed higher thresholds in preterm than in term neonates. At the second testing, the responses were equivalent in both groups.

The results of this research corroborate with other cohort studies using ASSR in the evaluation of term and preterm neonates.[37,38] On the other hand, the results of this study

Table 1 Means and standard deviation for ages in both groups during the first testing

Variables	Total sample ($n = 63$)	Preterm ($n = 33$)	Term ($n = 30$)	P-value
Age (days) – Averages ± SD [min – max]	15.3 ± 7.0 [6–28]	26.2 ± 3.1 [20–28]	8.7 ± 3.2 [6–15]	< 0.001*
GA (week) – Averages ± SD [min – max]	36.4 ± 2.2 [32–40]	34.6 ± 1.2*** [32–36]	38.7 ± 1.2 [37–40]	< 0.001*
Gender – n (%)				
Male	30 (47.6%)	15 (45.4%)	15 (50%)	0.776**
Female	33 (52.3%)	18 (54.5%)	15 (50%)	–

Abbreviations: GA, Gestational Age; SD, Standard Deviation.
* T-Student test to independent samples;** Chi-square of Pearson Test; *** Gestational age less than 37 weeks.

Table 2 Means and standard deviations for ASSR thresholds (dBNA) in both groups for 500, 1000, 2000, and 4000Hz during the first testing

ASSR Frequencies	Total sample (*n* = 63)	Preterm (*n* = 33)	Term (*n* = 30)	p*
	Average ± SD [min–max]	Average ± SD [min – max]	Average ± SD [min – max]	
500 Hz				
RE	38.9 ± 9.8	42.9 ± 8.4	34.7 ± 9.2	0.006
LE	38.3 ± 9.7	42.7 ± 8.1	34.7 ± 8.9	0.009
p**	0.413	0.276	1.000	
1000 Hz				
RE	28.7 ± 7.4	30.8 ± 7.1	25.2 ± 5.1	0.003
LE	28.3 ± 6.9	31.0 ± 6.6	25.4 ± 5.9	0.007
p**	0.137	0.714	0.101	
2000 Hz				
RE	26.1 ± 5.6	27.8 ± 6.8	22.8 ± 5.6	0.005
LE	25.2 ± 5.2	27.7 ± 6.5	22.9 ± 5.4	0.008
p**	0.213	0.102	0.674	
4000 Hz				
RE	26.5 ± 6.2	28.8 ± 6.3	23.7 ± 5.7	0.008
LE	26.9 ± 5.9	27.6 ± 6.2	23.6 ± 5.9	0.009
p**	0.831	0.773	0.771	

Abbreviations: ASSR, Auditory Steady-State Evoked Potential; dBNA, decibel hearing level; LE, left ear; RE, right ear; SD, Standard Deviation.
* T-Student Test to independent samples; ** T-Student Test to paired samples.

disagree with other research[6] in which the results showed no significant differences comparing the gestational age. However, methodological differences can justify this difference between studies, inasmuch as the electrophysiological assessment were performed in older preterm. One study[38] showed a decrease around 10dB in the preterm group with advancing age. In this study this difference was around 9dB and corroborate with the cited research.

Others studies with neonatal and pediatric population[9,31] reported ASSR' threshold around of 34dB at the frequency of 500Hz, 24.6 to 25.1 for 1000 Hz, 23.4 to 23.7 Hz for 2000, and 25.8 for 4000Hz. In this study, we found similar results for preterm at 18 months of age. These findings suggest that the preterm has a different way for the auditory maturational development on the brainstem.

Other studies[7,23,33] using a different electrophysiological assessment also described significant differences between term and preterm. For this research, the electrophysiological thresholds are higher in preterm than in term neonates. Another study[3] found different results, but this can be justified by the different methodology, in which the electrophysiological assessment took place with two months difference between term and preterm neonates. Based on the results from the present study, the hypothesis that electrophysiological thresholds changed with increasing age can be confirmed.

In this study, the comparison between ears showed no significant differences in both groups. This results are similar to those found in others studies.[15,23,33,39] On the other hand, others researchers found higher thresholds for the left ear.[39]

The comparison of genders also found no differences between groups. These results are similar with recent literature with ASSR[10,34,40] and with others research with ABR.[3,4,23,33] These results suggest that the audiological maturation occurs in a similar way in both genders, in term or preterm.

In this study, the thresholds of the frequency of 500Hz had higher values than the other frequencies in both groups. This finding corroborates with other research.[6,9,16,21,22,34] This occurs due to the interference of electrophysiological or environmental noise at low frequencies. Moreover, the cochlear tonotopy in which there is a decline for the amplitude due to the dispersion of energy in the cochlear apex may also explain the difference between frequencies.[6,9,16,21,22,34]

Based on our research, we can infer that the maturational process occurs in a different way for preterm neonates due to the immaturity of the auditory system. Because the electrophysiological thresholds are higher in preterm than in term neonates, we can infer the difference of neurofilament in the auditory pathways between the groups. This is important to the diagnosis of these groups. The higher threshold cannot be considered as hearing loss, as it is rather attributed to the auditory maturational process. Furthermore, the intrinsic development and environmental acoustic stimulation may have contributed to the improvement of neural synchrony for preterm neonates along the maturational process.

Table 3 Means and standard deviations for ASSR thresholds (dBNA) in both groups for 500, 1000, 2000 and 4000Hz during the 18th month of life

Frequencies tested by ASSR	Preterm ($n = 26$)	Term ($n = 30$)	p*
	Average ± SD [min – max]	Average ± SD [min – max]	
500 Hz			
RE	35.1 ± 7.2	34.7 ± 9.2	0.323
LE	34.9 ± 7.4	34.7 ± 8.9	0.385
p**	0.329	1.000	
1000 Hz			
RE	24.9 ± 5.8	25.2 ± 5.1	0.457
LE	24.1 ± 6.7	25.4 ± 5.9	0.528
p**	0.714	0.101	
2000 Hz			
RE	23.1 ± 5,9	22.8 ± 5.6	0.516
LE	23.2 ± 6,1	22.9 ± 5.4	0.497
p**	0.721	0.674	
4000 Hz			
RE	23.5 ± 6.2	23.7 ± 5.7	0.648
LE	23.9 ± 6.1	23.6 ± 5.9	0.598
p**	0.715	0.771	

Abbreviations: ASSR, Auditory Steady-State Evoked Potential; dBNA, decibel hearing level; LE, left ear; RE, right ear; SD, Standard Deviation. * T-Student Test to independent samples; ** T-Student Test to paired samples.

Table 4 Means and standard deviations for ASSR thresholds (dBNA) in both groups according to gender during the first testing

Frequencies tested by ASSR	Female ($n = 18$)	Male ($n = 15$)	p*
	Average ± SD	Average ± SD	
500 Hz			
RE	42.8 ± 8.6	42.8 ± 7.5	0.898
LE	42.7 ± 9.5	41.9 ± 8.7	0.768
1000 Hz			
RE	31.5 ± 6.4	30.8 ± 6.9	0.785
LE	31.2 ± 6.5	30.6 ± 7.8	0.771
2000 Hz			
RE	29.3 ± 6.9	27.1 ± 5.8	0.933
LE	27.7 ± 6.2	26.9 ± 5.3	0.911
4000 Hz			
RE	28.7 ± 6.7	28.6 ± 6.9	0.973
LE	28.9 ± 6.6	27.7 ± 6.7	0.981

Abbreviations: ASSR, Auditory Steady-State Evoked Potential; dBNA, decibel hearing level; LE, left ear; RE, right ear; SD, Standard Deviation. * T-Student Test to independent samples.

Table 5 Means and standard deviations for ASSR thresholds (dBNA) in both groups according to gender during the first testing

Frequencies tested by ASSR	Female ($n = 15$)	Male ($n = 15$)	p*
	Average ± SD	Average ± SD	
500 Hz			
RE	35.6 ± 6.8	32.7 ± 10.4	0.670
LE	34.7 ± 7.9	32.6 ± 9.8	0.476
1000 Hz			
RE	24.1 ± 5.1	24.8 ± 7.1	0.734
LE	25.9 ± 5.2	25.1 ± 6.5	0.653
2000 Hz			
RE	24.1 ± 5.1	23.2 ± 4.8	0.769
LE	24.3 ± 4.9	23.0 ± 5.1	0.691
4000 Hz			
RE	26.0 ± 5.9	26.2 ± 6.1	0.832
LE	25.9 ± 6.1	26.1 ± 6.8	0.782

Abbreviations: ASSR, Auditory Steady-State Evoked Potential; dBNA, decibel hearing level; LE, left ear; RE, right ear; SD, Standard Deviation. * T-Student Test to independent samples.

Conclusions

Preterm neonates have significantly higher thresholds at all frequencies at the first testing compared to term neonates. This difference was not found at 18 months, showing the auditory pathway maturation. The comparison between ears and gender found no difference in both groups.

The results of this study are relevant to audiological diagnosis of neonatal population, avoiding the false positives results. These findings help the audiologist to differentiate the results in the ASSR and show that the gestational age of the newborn at the time of evaluation should be considered.

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