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Original Paper

Auditory Steady-State Response in the First Six Months of Life

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Key Words

Auditory evoked potentials · Hearing · Brainstem

Abstract

Objective: To characterize the thresholds of the auditory steady-state response that relates to term newborns and infants. **Design:** The study was cross-sectional using auditory steady-state response assessment, and the real-ear-to-dial difference was measured in the external auditory canal. **Study Sample:** The study group included 60 newborns and infants between the age of 0 and 6 months. **Results:** A statistically significant difference was found in the carrier frequency variable for auditory steady-state response thresholds but not in comparison to ages. Furthermore, there is an association between auditory steady-state response thresholds and the real-ear-to-dial difference. **Conclusion:** The same threshold can be used as a normality reference for this age range, with distinct values for the different carrier frequencies. The influence of external auditory canal amplification should be taken into account.

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Introduction

With the continuous expansion of newborn hearing screening programs, a significant change in the profile of the population that comes for care in auditory health services is taking place. In order to provide precise answers about the hearing evaluation, language and speech therapists must define the presence or absence of hearing deficiency in an age group in which a child generally does not present motor, cognitive, and language development.

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Thus, in the process of infantile hearing evaluation, the Joint Committee on Infant Hearing [2007] recommends that procedures of behavior, electroacoustic, and electrophysiological evaluation be used to register and diagnose with precision the degree, configuration, and type of hearing loss up to 3 months of age.

Considering that the basis of the therapeutic process of a hearing-deficient child is the determination of the threshold, electrophysiological methods, such as the auditory brainstem response (ABR) generated by transient and continuous stimuli, are essential in infantile hearing evaluations since they make it possible to predict the psychoacoustic thresholds.

The assessment of the ABR with click or tone burst stimuli (i.e., a specific frequency) is already established in the literature. In the last decade, attention was paid to research on the auditory steady-state response (ASSR) in the pediatric population, with the use of tones modulated between 75 and 110 Hz, so that sleep does not interfere [Cohen et al., 1991; Lins and Picton, 1995], and a minor influence of the maturation process compared with the ASSR at 40 Hz [Ayogi et al., 1994].

The ASSR is evoked by a sound stimulus that generates electric activity in the auditory system, probably in the same region of the brainstem responsible for the generation of wave V and the SN10 (slow negative 10) in the ABR [Lins, 2002]. The fast sound stimulus prevents the nervous system from going back to the normal state of rest, thus generating an overlapping of continuous neural responses called the steady state, whose main characteristic is the same type of continuous sound stimulus presented to an individual [Stapells et al., 1984; Lins, 2002; Venema, 2004].

The advantages of the ASSR in comparison to the ABR are based on the possibility of carrying out research on the electrophysiological thresholds of binaural and multifrequential forms and on the objective analysis of the response, which does not decrease the responsibility of the professional in determining the threshold but, rather, decreases the risk of a subjective analysis. Another important aspect is the maximum intensity of the stimulus presentation, as strong as 120 dB HL, which makes it possible to characterize the child's residual hearing. However, it has been noted that artifacts or distortions may be present in the recording of ASSRs at high levels [Gorga et al., 2004].

Several studies that have been carried out with children and adults have shown a significant correlation of the thresholds that are considered by the ASSR to psychoacoustic thresholds [Dimitrijevic et al., 2002; Lins, 2002; Luts and Wouter, 2005; Schmulian et al., 2005]; however, the frequency of 0.5 Hz is of lesser correlation [Perez-Abalo et al., 2001; Swanepoel et al., 2004; Picton et al., 2005; Vander Werff and Brown, 2005; Van Maanen and Stapells, 2005]. Such a finding was not found by Duarte [2007] when using the exponential modulation for the amplitude associated with the frequency modulation.

However, proportionally few studies have been carried out with the neonate and breast-feeding population, which presents particularities in regard to the anatomophysiology of the auditory system and the maturation process of the structures [Rickards et al., 1994; Lins et al., 1996; Sávio et al., 2001; Rance and Rickards, 2002; John et al., 2004; Rance et al., 2005; Calil et al., 2006; Rance and Tomlin, 2006; Van Maanen and Stapells, 2009; Ribeiro et al., 2010].

Analyzing the electrophysiological response in this age group is of utmost importance since the immaturity of the peripheral structures, mainly the size of the external auditory canal (EAC), can directly affect the level of the auditory stimulus, thus offering bigger amplification in smaller EACs. The sound pressure level (SPL) inside the EACs of neonates/infants is modified by increasing the size of the EAC with respect to the person's growth as a whole. Thus, it is fundamental to determine the magnitude of this amplification that occurs in the EAC for an accurate analysis of the ASSR threshold.

The accomplishment of measurements with a probe microphone and insertion phones and calculating the difference between the intensity of the presented stimulus in the dial of

the equipment and the real SPL in the auditory canal – that is, the real-ear-to-dial difference (REDD) – supplies information about the real SPL that is detected in the neonate's/breastfed infant's auditory canal by means of insertion phones. Such an approach makes it possible to verify how the acoustic modifications of this cavity interfere with the intensity of the stimulus.

Considering what has been said, the objective of the present study was to characterize the ASSR thresholds in term neonates and infants to investigate the influence of the maturation process in the electric response of the auditory system.

Materials and Methods

A transversal study was carried out at the Audiology and Speech Pathology Clinic, University of São Paulo, Bauru campus, Brazil, with the approval of the Ethics Committee on Research in Humans of the Dentistry School under the process No. 122/2010.

Participants

Neonates and infants with a gestational age ≥ 37 weeks without any risk factors for hearing deficiency were defined by the Joint Committee on Infant Hearing [2007]. The study group consisted of 60 participants who were divided into three groups according to their ages: group I – from 0 to 1 month and 29 days ($n = 20$); group II – from 2 to 3 months and 29 days ($n = 20$), and group III – from 4 to 6 months ($n = 20$). The inclusion criteria in the present study were defined for each group as reported below.

Group I (from 0 to 1 Month and 29 Days)

In group I, the present result of 'pass' in the newborn hearing screening was defined as follows: transient-evoked otoacoustic emissions with a signal/noise ratio ≥ 6 dB and a reproducibility of $\geq 70\%$ in three nonconsecutive frequency bands, including 4 kHz, with a peak click stimulus of 80 dB SPL. Tympanometry with a probe of 1 kHz was carried out on the day of the study of the ASSRs, considering the middle ear normal when a tympanometric curve with a maximum peak admittance of up to 0.6 mmho [Margolis et al., 2003] or a curve classified according to the Marchant method [1986], adapted by Baldwin [2006], were associated with normal otoscopy findings by an ENT doctor.

Group II (from 2 Months to 3 Months and 29 Days) and Group III (from 4 Months to 5 Months and 29 Days)

In groups II and III, in addition to the 'pass' result in the newborn hearing screening, the transient-evoked otoacoustic emissions would have to be present before the assessment of the ASSR – that is, a signal/noise ratio of ≥ 3 dB and a reproducibility $\geq 50\%$ in the frequency bands of 1, 2, 3, 4, and 5 kHz, with a peak of the click stimulus at 80 dB SPL. The presence of otoacoustic emissions with normal amplitude was considered suggestive of the functionality of the external and/or middle ear.

Neural integrity was considered to be present when ASSRs were recorded in the frequencies of 0.5, 1, 2, and 4 kHz at 60 dB nHL.

Parameters of Stimulus

The ASSR was determined by using the MASTER (multiple auditory steady-state response) system, version 2.04.i00, from Bio-Logic Systems Corp. Pure tones were used in the carrier frequencies of 0.5, 1, 2, and 4 kHz, modulated 100% in amplitude and 20% in frequency, with the modulation frequencies proposed in the protocol of the equipment being 91,406, 93,750, 96,094, and 98,437 Hz for the presented stimuli of the monaural form by means of

the insertion phone ER-3A. The sequence of the start of the research was interspersed in accordance with the ear to discard the influence of this variable in the analysis of the data.

Parameters of Recording and Analysis of the ASSR

Disposable electrodes with gel were used, positioned in Fz (active electrode), Oz (reference), and Fpz forehead (ground). Their impedance was kept below 5 k Ω and the impedance balance of the interelectrodes was lower than, or equal to, 2 k Ω . The recording of the EEG was conducted by using a gain of 50,000, a 1- to 300-Hz band-pass filter, 6 dB/octave, and a digital/analogical conversion speed of 1 kHz. Each sweep consisted of 16 epochs containing 1,024 points each, which were constantly analyzed and rejected when the value of the amplitude exceeded ± 40 μ V.

Procedures

The examination was carried out in an acoustic booth while the child was naturally sleeping. All of the procedures were conducted within a single session or two.

The assessment of the electrophysiological threshold was carried out at an intensity of 60 dB HL at first, followed by an intensity of 30 dB HL with a posterior decrease of 10 dB up to an intensity at which no response was obtained ($p \geq 0.1$). The assessment of the thresholds in the frequencies that did not present a response was redone, keeping the multifrequential mode in the intermediate intensities.

A response was considered to occur when there was a significant result ($p \leq 0.05$) in at least four consecutive sweeps, that is at least half of the forecasted sweeps. In the case of an absence of a response, the total number of sweeps forecasted for the particular intensity was presented. The smaller intensity was 0 dB HL, which was considered the threshold when the response was at the significance level $p \leq 0.05$ [Ribeiro et al., 2010].

To classify the obtained responses as positive or false positive, the criteria determined by Duarte [2007] were used:

- a threshold that was present but preceded by two or more absences of responses was considered absent or false positive;
- a present threshold that was preceded by the absence of a response or a questionable threshold was considered present, that is, a real threshold;
- a questionable threshold that was preceded by the presence of a response was considered present;
- two consecutive questionable thresholds that were preceded by a present threshold were considered present.

Each sweep was automatically analyzed by using the frequency spectrum resulting from the fast Fourier transform, which consists of the verification of the amplitude of the ASSR at the frequency of a specific modulation compared with the adjacent ones (60 bins of frequency below and 60 bins of frequency above the response). It was then analyzed by using the F test with a confidence interval of 95% [Lins and Picton, 1995].

In situ Stimulus Calibration

To determine the difference between the intensity of the stimulus presented in the dial of the equipment and the real SPL measured in the auditory canal – that is, the REDD – the Hearing Aid Analyzer FONIX FP 35 (Frye Electronics) equipment was used, which comprises a system of double microphones (probe and reference) and a speaker connected to a computer. For the measurement, the speaker was deactivated so that the system of microphones caught only the sound emanating from an external source, in this case from the MASTER system.

For the monitoring, a flexible silicone pipe of 1.1 mm was introduced in the EAC and placed on the phone with plastic film. Its positioning was established according to the size of

Table 1. Comparative statistical analysis for the REDD values and the ASSR thresholds for the frequencies of 0.5, 1, 2, and 4 kHz

Frequencies, kHz	Mean REDD, dB SPL ¹	Mean ASSR, dB HL ²	Group	Mean REDD, dB SPL ³	Mean ASSR, dB HL ⁴
0.5	10.64A	38.83A	I	12.08a	37.00a
			II	9.39a	38.75a
			III	10.45a	40.75a
1	13.742B	26.00B	I	14.78a	23.50a
			II	12.95a	30.50a
			III	13.49a	24.00a
2	20.77C	15.58C	I	20.54a	17.75a
			II	21.09a	14.75a
			III	20.69a	14.25a
4	16.95D	20.83D	I	19.73a	22.25a
			II	16.72b	19.75a
			III	14.40b	20.50a

¹ Among the frequency levels, just for REDD, means followed by different capital letters indicate a statistically significant difference.

² Among the frequency levels, just for ASSR, means followed by different capital letters indicate a statistically significant difference.

³ Within each frequency level, just for REDD, means followed by different lowercase letters indicate a statistically significant difference.

⁴ Within each frequency level, just for ASSR, means followed by different lowercase letters indicate a statistically significant difference.

the baby's auditory canal by age group (3–5 mm beyond the phone), in accordance with the standards outlined by Keefe et al. [1993].

The SPL that was generated inside the child's EAC by the presented stimulus was obtained at the beginning of the test for the modulated stimuli at the carrier frequencies of 0.5, 1, 2, and 4 kHz at an intensity of 60 HL, the same ones used for the assessment of the ASSR.

Data Analysis

A descriptive analysis of the data obtained by means of the measurements of central tendency, such as the average and the measurement of variability by standard deviation, was conducted. The inferential analysis was carried out by means of the multivariate analysis of variance test. The frequencies of 0.5, 1, 2, and 4 kHz were applied as repeated measurements and the age groups (group I, group II, and group III) were used as factor groups. The Huynh-Feldt epsilon was used to adjust repeated measurements when necessary. A significance level of ≤ 0.05 (or 5%) was adopted for the rejection of the null hypothesis, as recommended for biological assays.

Results

The statistical analysis demonstrated that, for the REDD values, there is a significant difference only in the frequency of 4 kHz when comparing group I with group II and group III. Moreover, there was no statistically significant difference between the groups regarding the

Table 2. Descriptive statistical analysis for the REDD values and the ASSR thresholds for the frequencies of 0.5, 1, 2, and 4 kHz

Frequency, kHz	REDD				ASSR			
	mean	SD	95% CL		mean	SD	95% CL	
			lower	upper			lower	upper
0.5	10.64	0.76	9.12	12.16	38.83	1.71	35.41	42.25
1	13.74	0.52	12.70	14.80	26.00	1.43	23.13	28.87
2	20.78	0.42	19.94	21.61	15.58	1.0	13.58	17.58
4	16.95	0.45	16.04	17.86	20.83	1.05	18.72	22.94

Table 3. Correlation between the REDD values and the ASSR thresholds for the frequencies of 0.5, 1, 2, and 4 kHz

Frequency, kHz	p value
0.5	0.058
1	0.063
2	0.113
4	0.096

ASSR thresholds (table 1). Thus, for the following analyses, the age factor was not considered and a single group was analyzed.

The descriptive statistical analysis of the REDD values and the ASSR thresholds in the evaluated frequencies can be found in table 2. The correlation between the REDD values and ASSR thresholds for the frequencies of 0.5, 1, 2, and 4 kHz is described in table 3. Figure 1 demonstrates the cumulative percentage of the presence of the ASSR for each assessed carrier frequency in the evaluated group. In figure 2, the quadratic polynomial model demonstrating the behavior of the ASSR thresholds and the REDD values for the analyzed frequencies can be found.

Discussion

In the last few decades, the literature directed towards infantile audiological evaluation has presented research on ASSR as a promising method because of the numerous advantages of this procedure. However, its clinical application is still very restricted. In contrast to the ASSRs with click and tone burst stimuli that have already been widely studied, little is known about the effect of the maturation process of the peripheral and central auditory system on the ASSR thresholds and, consequently, on the standard of normality to be used for the different age groups.

It is known that the volume of the EAC increases significantly with age, due to change in the size of the structure [Keefe et al., 1993], with direct implications for the SPL of the sound that will stimulate the cochlea. The smaller the EAC, the bigger the amplification of the SPL of the presented stimulus will be [Simonetti, 2004]. Thus, knowing that the magnitude of this amplification is fundamental, regardless of the type of transducer that is used, it can be observed that, when calibrating stimuli in couplers, the intensity level that is really stimulating the auditory system of the child is underestimated, especially for click and tone burst stimuli starting at 4 kHz [Sininger et al., 1997].

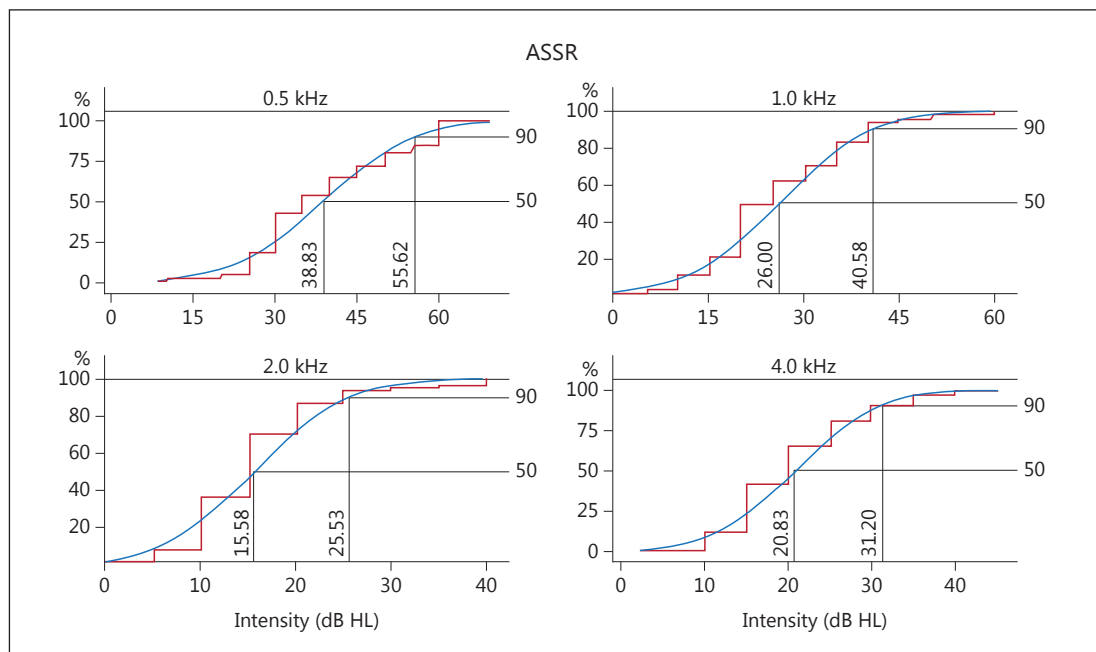


Fig. 1. Cumulative response for 50 and 90% of the ASSR thresholds at the frequencies of 0.5, 1, 2, and 4 kHz.

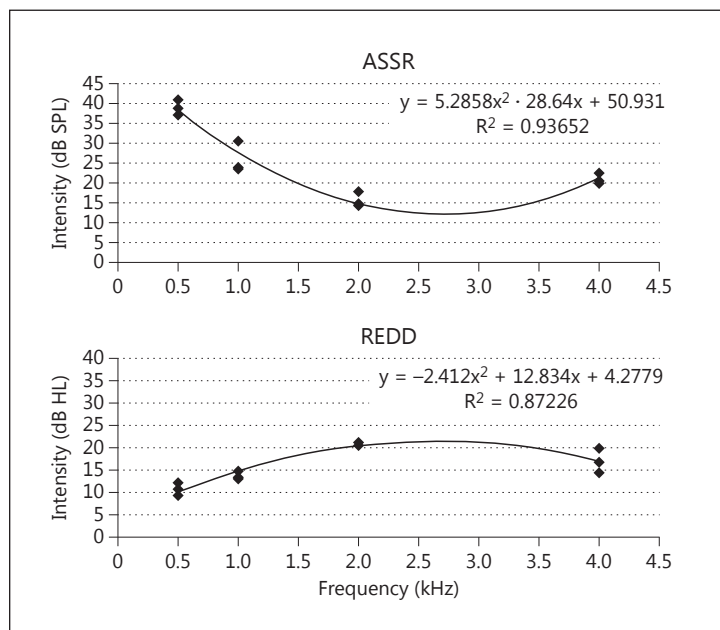


Fig. 2. Quadratic polynomial model demonstrating the behavior of the ASSR thresholds and the values of REDD, considering the analyzed frequencies.

Thus, some studies assumed a methodology to measure in situ the level of intensity of the stimulus for the analysis of the ASSR thresholds. However, works that have measured the variation of the SPL of the stimulus in the EAC (REDD) of neonates and infants have not been found in the literature; rather, the difference in the level of intensity of the stimulus obtained in the coupler and that obtained in situ was measured. In spite of the difference in methods,

it is possible to engage in a comparative discussion between the studies when the amplification characteristics that occur in the EAC are considered.

In the present study, the variation of the REDD in accordance with age was observed only for the frequency of 4 kHz when group I was compared to group II and group III (table 1). The occurrence of bigger amplifications in the EAC in the first 2 months of life was described by Rance and Tomlin [2006] and Bingham et al. [2009]. However, in this last study, this fact was evidenced only at the frequencies of 1.5 and 2 kHz. With regard to the finding for the frequency of 4 kHz, it is known that this frequency exactly resonates with the EAC for the age group of 1 month. This is evidenced by the findings of Keefe et al. [1994], describing that the resonance of the EAC can be found in the frequency band of 2–6 kHz for the age group of 1–24 months, with a level of 4.4 kHz at the age of 1 month and reaching 2.9 kHz at the age of 24 months. These data can justify the obtained results, since the biggest amplification occurs at the frequency of resonance of the EAC.

In the REDD analysis, considering the total number of neonates and infants evaluated, the frequency was demonstrated to be a determinant variable because there was a statistically significant difference in the REDD values obtained for 0.5, 1, 2, and 4 kHz (tables 1, 2). Bigger amplification was noted in the EAC at the 2-kHz frequency, followed by 4, 1, and 0.5 kHz. The studies differ in regard to the decreasing distribution of the frequencies considering the magnitude of the amplification. However, in general, there is a consensus that, in the first years of life, greater amplification occurs at high frequencies than at low frequencies [Rickards et al., 1994; Lins et al., 1996; Rance and Rickards, 2002; John et al., 2004; Sávio et al., 2001; Calil et al., 2006; Rance and Tomlin, 2006; Van Maanen and Stapells, 2009; Ribeiro et al., 2010].

With regard to the ASSR thresholds, no statistically significant difference was observed between the three age groups; that is, the anatomical modifications of the peripheral auditory system and the possible maturation process of the central auditory structures that occur in the first 6 months of life were not reflected in the ASSR thresholds. Among the studies conducted with similar age groups, some presented similar results [Rance and Tomlin, 2006; Van Maanen and Stapells, 2009]. Others found that age is an important variable, even when the first months of life [Sávio et al., 2001; John et al., 2004] or a wider age group [Levi et al., 1993; Rickards et al., 1994; Cone-Wesson et al., 2002; John et al., 2004; Luts et al., 2006; Rance and Tomlin, 2006] were analyzed. However, the findings of the present study suggest that, in clinical practice, in the first 6 months of life, the same ASSR threshold can be used as a reference of normality.

In figure 1, which presents the ASSR thresholds estimated at 50% in the regression function, it is observed that the threshold is raised at the frequency of 0.5 kHz and that there is a reduction as the frequency increases; such a finding is similar to the one described by Van Maanen and Stapells [2009]. However, the frequency of 2 kHz had the smallest threshold [Lins et al., 1996; Rance and Rickards, 2002; John et al., 2004].

In this study, the thresholds of 50% that were obtained were 35, 25, 15, and 20 dB HL at the frequencies of 0.5, 1, 2, and 4 kHz, respectively. The majority of the study group, i.e. 90%, presented thresholds at 60, 40, 25, and 30 dB HL, with the same sequence of frequencies. Although there are studies that have evaluated the ASSR in neonates and infants, the comparative discussion of the thresholds is difficult due to the wide variety of methodology that was employed, involving different types of equipment, frequencies of modulation, units of calibration of the stimulus (SPL or hearing level), time of stimulus, and test environments.

However, there is consensus when demonstrating that the frequency of 0.5 kHz is the one that presents a greater threshold [Rickards et al., 1994; Lins et al., 1996; Rance and Rickards, 2002; John et al., 2004; Calil et al., 2006; Rance and Tomlin, 2006; Van Maanen and Stapells,

2009; Ribeiro et al., 2010]. Possible explanations for such findings include the smaller neural synchrony for this frequency [Lins et al., 1996; Rance et al., 2005], maturational changes of the external/middle ear [Naeve et al., 1992; Keefe et al., 1993], and the existence of higher levels of noise at this frequency [Rickards et al., 1994].

No significant correlation between the REDD values and the ASSR thresholds (table 3) was observed; that is, greater amplifications in the EAC did not necessarily imply smaller thresholds, considering each analyzed frequency. However, an association between the variables can be observed by means of a quadratic polynomial model, as shown in figure 2, which has a significant coefficient of determination R^2 ($p < 0.05$). The threshold of the ASSR decreases for the interval of 0.5–2.5 kHz and grows when the frequency increases to 4 kHz. In the REDD, the inverse occurs: when the frequency increases from 0.5 to 2.5, the amplification in the EAC also increases. Conversely, from the frequency of 2.5 kHz, it decreases.

Thus, an influence of the amplification in the EAC on the threshold of the ASSR in the studied age group cannot be excluded, as described by Rance and Tomlin [2006] and Van Maanen and Stapells [2009]. This outcome indicates that immaturity in the neural synchrony that would raise the threshold in neonates can be compensated for by the bigger SPL, due to the amplification in the EAC in this population. It must be highlighted that the audiologist should consider that the intensity supplied in the dial of the equipment does not precisely reflect the intensity level that stimulates the auditory system of neonates and breastfed infants. Thus, the ASSR thresholds that were obtained during audiological evaluation supply information on normality and the degree of the sensorineural hearing loss. However, they should not be used as a single value in the process of regulating a personal sound amplification device, which strengthens the need for complementary in situ measurements.

Conclusion

The characterization of the ASSR thresholds in the first 6 months of life demonstrated that one threshold can be used as a reference of normality for this age group, with distinct values for the different carrier frequencies. The influence of the amplification in the EAC on the ASSR thresholds must be considered because there is the possibility that it will mask the influence of the maturation process on the electric response of the auditory system.

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Disclosure Statement

The authors report no conflict of interest.

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