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The influence of gender on brainstem auditory evoked potentials' responses to different stimuli in newborns

Influência do sexo nas respostas do potencial evocado auditivo de tronco encefálico com diferentes estímulos em neonatos

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ABSTRACT

Purpose: To evaluate the influence of gender on the brainstem auditory evoked potentials V-wave latency and amplitude values in newborns, with different stimuli. **Methods:** 62 full-term newborns (29 females and 33 males) participated in this study. The electrophysiological threshold of the brainstem auditory evoked potential was investigated with four different stimuli – click, broadband (BB) Ichirp, tone-burst, and specific-frequency (SF) Ichirp –, in intensities of 60, 40 and 20 dBnHL. The genders were compared in each stimulus and intensity. **Results:** The results obtained showed lower latency and greater amplitude in females for the click stimulus. However, for tone-burst, the females presented higher latency and greater amplitude. When the BB-Ichirp and SF-Ichirp stimuli were used, the gender did not present a statistically significant difference in the latency and amplitude values. **Conclusion:** The BAEP V-wave in newborns is influenced by gender when the click and tone-burst stimuli are used. However, such influence was not noted when the BB-Ichirp and SF-Ichirp stimuli were used.

Keywords: Auditory evoked potentials; Brainstem auditory evoked potentials; Hearing; Newborn; Electrophysiology.

RESUMO

Objetivo: avaliar a influência da variável sexo nos valores da latência e amplitude da onda V do potencial evocado auditivo de tronco encefálico, com diferentes estímulos em neonatos. **Métodos:** participaram deste estudo 62 neonatos nascidos a termo (29 do sexo feminino e 33 do sexo masculino). Realizou-se a pesquisa de limiar eletrofisiológico do potencial evocado auditivo de tronco encefálico com quatro estímulos diferentes (clique, *Ichirp* banda larga-BL, *tone burst* e *Ichirp*-frequência específica-FE), nas intensidades de 60, 40 e 20 dBnNA. A variável sexo foi comparada para cada estímulo e intensidade. **Resultados:** os resultados obtidos demonstraram menor latência e maior amplitude no sexo feminino para o estímulo clique. Entretanto, para o estímulo *tone burst*, o sexo feminino apresentou maior latência e maior amplitude. Quando utilizados os estímulos *Ichirp*-BL e *Ichirp*-FE, a variável sexo não apresentou diferença estatisticamente significativa para os valores de latência e amplitude. **Conclusão:** a onda V do PEATE de neonatos sofre influência da variável sexo, quando utilizados os estímulos clique e *tone burst*. Entretanto, não houve tal influência quando utilizado o estímulo *Ichirp* banda larga–BL e o estímulo *Ichirp* frequência específica-FE.

Palavras-chave: Potenciais evocados auditivos; Potencial evocado auditivo de tronco encefálico; Audição; Neonato; Eletrofisiologia.

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INTRODUCTION

The audiological diagnosis in newborns must be conducted through objective and precise methods, including the physiological and electrophysiological hearing measures[1,2]. The brainstem auditory evoked potentials (BAEP) – which is considered the gold standard assessment for the newborn population to estimate their auditory thresholds and diagnose the integrity of their auditory pathway to the brainstem – are included in these measures[3-10].

Picking up and recording BAEP responses can be done with different acoustic stimuli[3,5], which include the click, broadband Ichirp, tone-burst, and specific-frequency Ichirp stimuli. The click stimulus is currently the most used in clinical practice, especially in analyzing the integrity of the auditory pathway to the brainstem. Concerning the assessment of the electrophysiological threshold, this is not the ideal stimulus to use, since with the click the sound wave runs through a large cochlear region and takes a considerable time to reach the base of the cochlea[3]. As a larger region of the cochlea is stimulated, the click leads to non-specified frequencies – their specificity, though, is very important in the search for thresholds in newborns and small children, who do not yet respond to a behavioral audiological assessment[3,11].

The stimulus used in clinical practice to estimate auditory thresholds is the tone-burst (TB), which enables specific-frequency (SF) bands of the cochlea to be stimulated, thus making it possible to determine more precisely the individual's audiometric configuration[12,13].

Moreover, regarding the different stimuli used when conducting the BAEP, the chirp has been arousing the scientific community's interest[14-16]. It is known that the use of this stimulus makes better neural synchrony possible, as it simultaneously stimulates the whole cochlea, enabling greater synchrony when compared with the other stimuli[17]. Due to its physical characteristics, the electrophysiological waves produced with the chirp stimulus reach greater amplitudes and better morphologies in the V-wave[11,14-16]. Other authors have verified the difference between the chirps and the click precisely in triggering the stimulation[14,17].

It should be noted that various chirp stimuli were tested and proposed by different researchers and companies that produce the equipment to record and analyze the evoked potentials. Such stimuli can have different physical characteristics depending on the maker, and in the literature consulted different names were found for the different chirps, as, for instance, M-chirp[18], CE-chirp® and NB CE-chirp®[19], LS-chirp[20], M-chirp and A-chirp[21], and Ichirp[22]. The Ichirp stimulus was developed by the Intelligent Hearing Systems® (IHS) team, one of the main manufacturers of audiological diagnosis equipment. It is believed that all different chirp stimuli behave similarly when stimulating the cochlea.

The Ichirp stimulus can be used as either broadband (BB) or specific frequency (SF). The difference between them is in how the frequencies are surveyed, as the broadband stimulates a larger area of the cochlea[10,16], whereas the specific frequency individually stimulates, for example, regions related to the 500, 1000, 2000 and 4000 Hz frequencies[7,20].

In the literature consulted, the findings of the BAEP in newborns were conflicting, when the V-wave latency values were compared between the genders. While some scholars[21,23-26] concluded that there are differences between the genders when using the CE-chirp, A-chirp, tone-burst and click stimuli, other stimuli did not present such differences[10,11,14,27,28]. The researchers who observed a difference between the genders found increased latencies for the males[23,25] and attributed this finding to the anatomical differences there are between the genders.

Due to the conflicting BAEP results regarding differences between the genders, an interest in studying the influence of gender with different stimuli arose.

The hypothesis in this research was that the difference in the maturation of the auditory pathway, as well as the anatomical differences observed by some authors when comparing the genders, can influence the BAEP electrophysiological responses. Hence, studying gender when recording the BAEP with different stimuli contributes to clinical practice, as it identifies the stimuli that present the least influence of the variable. Thus, this study sought to assist in the clinical reliability of the BAEP. It should also be highlighted that in the literature consulted no studies with such an Ichirp-related analysis were found, which reinforces the importance of this research.

Therefore, this study aimed at investigating the influence of gender on the BAEP V-wave absolute latency and amplitude values with different stimuli in full-term newborn children.

METHODS

This is an observational descriptive quantitative cross-sectional study, approved by the Human Research Ethics Committee – HREC – UFSM, under number 23081.032787/2017-78; it also fully complied with Resolution no. 466/12, which refers to research carried out with humans. Those responsible for the newborns were previously informed about the purpose and procedures involved in this study, to which all agreed, signing the Informed Consent Form (ICF).

The following inclusion criteria were defined to compose the sample: male and female 1- to 29-day-old newborns whose response passed in the neonatal auditory screening (NAS), indicating that transient evoked otoacoustic emissions (TEOAE) were present in both ears, with no risk factors for infant hearing loss. Furthermore, the newborns needed to be in proper conditions (fed and naturally sleeping) when the procedure proposed in this study was conducted. The newborns that did not conclude the procedure because of the parents' unquietness and/or physical tiredness were excluded from the sample.

Thus, 62 full-term newborns – 29 females and 33 males – participated in this study.

The data was collected through BAEP, using the Smart-EP module equipment made by Intelligent Hearing Systems®. In picking up this potential, the click, broadband Ichirp, tone-burst, and specific-frequency Ichirp stimuli were used. To keep the newborns from growing tired and samples from being lost, the methodological strategy used was to choose subjects randomly to record BAEP with the different stimuli. This way, the sample was randomized by type of stimulus. Hence, of the 62 newborns included in the sample, 30 (11 females and 19 males) performed BAEP with the click and broadband Ichirp stimuli, while 32 newborns (18 females and 14 males) performed BAEP with the tone-burst and specific-frequency stimuli.

For BAEP recording, the newborns were comfortably accommodated on the parent's/guardian's lap, naturally sleeping throughout the procedure. At first, the skin was sanitized with NuPrep® paste, and then the electrodes were fixed – the reference ones placed on the right (M2) and left mastoid (M1), and the active (Fz) and ground (Fpz) electrodes on the forehead. The recording started only after the impedance of the electrodes was under 3 kΩ.

The parameters used for all the stimuli were 2,048 stimuli in rarefied polarity, at a presentation rate of 27.7 stimuli per second, a band-pass filter of 100 to 3000 Hz. Windows of 24 milliseconds (ms) were used for the click, broadband Ichirp, specific-frequency Ichirp, and tone-burst stimuli. The stimulation was monaurally presented through ER-3A insert earphones in the intensities of 60, 40 and 20 dB HL. Each recording was repeatedly used to trace the V-wave in the stimuli researched, to ensure the repeatability and trustworthiness of the waves. During BAEP recording, the artifact rate of up to 10% of the total stimuli presented was accepted.

All the stimuli started in the intensity of 60 dB HL and were then presented in 40 and 20 dB HL. The ears were randomly chosen. BAEP recording with the tone-burst and SF-Ichirp stimuli was researched in two frequencies; in some newborns, the frequencies researched were 500 and 2000 Hz, while in others, 1000 and 4000 Hz. The option for the two-frequency research meant to avoid fatiguing the newborn. It should be noted that the procedure was conducted in one single appointment, and all the newborns were naturally sleeping, without the use of any sort of sedation.

To ensure trustworthy responses, all the BAEP recordings were independently analyzed by three judges, two of whom were speech-language-hearing pathologists and one, an otorhinolaryngologist. These judges received a copy of the tracings without the due markings and inserted the visual identification of the V-wave, considering their theoretical and practical experience in electrophysiology of hearing. This tracing analysis was blindly conducted. The third judge was only called when the judges who had analyzed the tracings disagreed. It should be highlighted that the third judge's intervention was necessary for only two tracings.

As the V-wave identification criterion for latency measures, the judges considered the positive peak preceding the greatest negative deflection. In its turn, the amplitude measure was obtained through the difference between the V-wave positive and negative peaks.

To analyze the comparison of the BAEP V-wave absolute latency and amplitude values between the genders, the results obtained were inserted in a spreadsheet editor. The statistical analyses started with the Shapiro-Wilk test, used to determine the data distribution (either normal or not) for each stimulus. The normal data distribution of all stimuli studied was verified. The Student's *t*-test was used for the comparison analysis.

The research considered the significance level of 5% in the statistical analyses. Throughout the study, the confidence intervals were developed with 95% statistical confidence.

RESULTS

Before analyzing gender, the ears assessed were compared. No statistically significant differences were observed between the ears for the click ($p = 0.853$), broadband Ichirp ($p = 0.756$), specific-frequency Ichirp ($p = 0.875$), and tone-burst stimuli ($p = 0.768$). Such analysis enabled, then, the gender analysis to be conducted considering the mean between the ears.

When comparing the BAEP V-wave absolute latency with the click stimuli, values numerically smaller for females were observed, with statistically significant differences in the intensities of 60 and 40 dBnHL. For the amplitude, there was a statistically significant difference in the intensity of 20 dBnHL, with greater amplitude noted for the females (Table 1).

As for the BAEP V-wave absolute latency and amplitude values with the presentation of broadband Ichirp stimulus, no statistically significant differences were found between the genders (Table 2).

Table 1. Analysis of latency in milliseconds (ms) and amplitude in microvolts (μV) of the V-wave in the brainstem auditory evoked potentials recorded with the click stimulus in different intensities, between males and females (n = 30)

Click Latency		Mean	Standard Deviation	CI	P-value	Click Amplitude	Mean	Standard Deviation	CI	P-value
Intensity 60	Female	7.13	0.20	0.08	0.005*	Female	0.21	0.07	0.03	0.140
	Male	7.31	0.25	0.08		Male	0.18	0.06	0.02	
Intensity 40	Female	7.85	0.33	0.14	0.045*	Female	0.15	0.05	0.02	0.363
	Male	8.04	0.35	0.11		Male	0.14	0.04	0.01	
Intensity 20	Female	8.84	0.46	0.19	0.297	Female	0.11	0.05	0.02	0.041*
	Male	8.96	0.42	0.13		Male	0.09	0.04	0.01	

Statistical test: Student's *t*-test; *p-value with a statistically significant difference
Subtitle: n = number of subjects

Table 2. Analysis of latency in milliseconds (ms) and amplitude in microvolts (μV) of the V-wave in the brainstem auditory evoked potentials recorded with the broadband Ichirp stimulus in different intensities, between males and females (n = 30)

BB Chirp Latency		Mean	Standard Deviation	CI	P-value	Chirp Amplitude	Mean	Standard Deviation	CI	P-value
Intensity 60	Female	9.87	0.40	0.17	0.086	Female	0.22	0.07	0.03	0.920
	Male	10.07	0.43	0.14		Male	0.22	0.08	0.02	
Intensity 40	Female	10.98	0.38	0.16	0.071	Female	0.23	0.08	0.03	0.485
	Male	11.15	0.32	0.10		Male	0.21	0.08	0.02	
Intensity 20	Female	11.98	0.38	0.16	0.167	Female	0.16	0.04	0.02	0.108
	Male	12.13	0.40	0.13		Male	0.14	0.05	0.02	

Statistical test: Student's *t*-test
Subtitle: n = number of subjects; BB = broadband; CI = confidence interval

Table 3. Analysis of latency in milliseconds (ms) and amplitude in microvolts (μV) of the V-wave in the brainstem auditory evoked potentials recorded with the specific-frequency tone-burst stimulus in different intensities, between males and females (n = 32)

SF Tone-Burst Latency			Mean	Standard Deviation	CI	P-value	SF Tone-Burst Amplitude	Mean	Standard Deviation	CI	P-value
500 Hz	Intensity 60	Female	7.37	0.45	0.23	0.516	Female	0.09	0.10	0.03	0.227
		Male	7.52	0.77	0.35		Male	0.08	0.08	0.02	
	Intensity 40	Female	8.57	0.29	0.15	0.317	Female	0.08	0.09	0.03	0.019
		Male	8.79	0.78	0.36		Male	0.08	0.09	0.02	
	Intensity 20	Female	10.16	0.68	0.36	0.309	Female	0.05	0.05	0.02	0.522
		Male	10.39	0.55	0.26		Male	0.06	0.05	0.03	
1000 Hz	Intensity 60	Female	7.80	0.71	0.30	0.886	Female	0.106	0.110	0.039	0.234
		Male	7.84	0.41	0.25		Male	0.126	0.125	0.049	
	Intensity 40	Female	9.06	0.78	0.33	0.637	Female	0.091	0.090	0.035	0.499
		Male	9.20	0.62	0.38		Male	0.100	0.110	0.028	
	Intensity 20	Female	10.41	0.75	0.31	0.390	Female	0.077	0.065	0.041	0.777
		Male	10.68	0.97	0.60		Male	0.081	0.075	0.031	
2000 Hz	Intensity 60	Female	7.41	0.41	0.21	0.475	Female	0.129	0.120	0.045	0.903
		Male	7.53	0.45	0.21		Male	0.127	0.125	0.048	
	Intensity 40	Female	8.67	0.66	0.35	0.618	Female	0.112	0.110	0.047	0.676
		Male	8.78	0.53	0.24		Male	0.106	0.105	0.041	
	Intensity 20	Female	10.08	0.85	0.45	0.806	Female	0.083	0.090	0.029	0.538
		Male	10.01	0.67	0.31		Male	0.077	0.090	0.027	
4000 Hz	Intensity 60	Female	7.94	0.56	0.24	0.073	Female	0.112	0.110	0.021	0.012*
		Male	7.55	0.51	0.32		Male	0.092	0.090	0.016	
	Intensity 40	Female	9.25	0.61	0.26	0.049*	Female	0.088	0.095	0.024	0.007*
		Male	8.80	0.47	0.29		Male	0.061	0.055	0.026	
	Intensity 20	Female	10.52	0.62	0.26	0.041*	Female	0.074	0.070	0.026	0.265
		Male	10.06	0.37	0.23		Male	0.138	0.050	0.268	

Statistical test: Student's *t*-test; *p-value with a statistically significant difference
Subtitle: n = number of subjects; SF = specific frequency; CI = confidence interval

Table 4. Analysis of latency in milliseconds (ms) and amplitude in microvolts (μV) of the V-wave in the brainstem auditory evoked potentials recorded with the specific-frequency Ichirp stimulus in different intensities, between males and females (n = 32)

SF Chirp Latency			Mean	Standard Deviation	CI	P-value	SF Chirp Amplitude	Mean	Standard Deviation	CI	P-value
500 Hz	Intensity 60	Female	8.03	0.78	0.41	0.950	Female	0.18	0.18	0.05	0.409
		Male	8.02	0.61	0.28		Male	0.16	0.16	0.05	
	Intensity 40	Female	9.59	1.33	0.70	0.956	Female	0.15	0.15	0.04	0.250
		Male	9.57	0.97	0.45		Male	0.13	0.12	0.05	
	Intensity 20	Female	11.17	1.54	0.81	0.687	Female	0.09	0.09	0.04	0.254
		Male	10.98	1.06	0.49		Male	0.07	0.07	0.02	
1000 Hz	Intensity 60	Female	8.22	0.95	0.40	0.096	Female	0.219	0.190	0.070	0.344
		Male	7.67	0.49	0.31		Male	0.190	0.145	0.094	
	Intensity 40	Female	9.62	0.93	0.39	0.064	Female	0.163	0.140	0.072	0.823
		Male	8.94	0.63	0.39		Male	0.157	0.145	0.071	
	Intensity 20	Female	11.35	1.18	0.50	0.002*	Female	0.117	0.095	0.058	0.196
		Male	9.99	0.62	0.39		Male	0.193	0.100	0.259	
2000 Hz	Intensity 60	Female	8.01	0.50	0.26	0.752	Female	0.186	0.180	0.046	0.906
		Male	7.95	0.46	0.21		Male	0.184	0.180	0.059	
	Intensity 40	Female	9.11	0.57	0.30	0.905	Female	0.142	0.150	0.054	0.870
		Male	9.14	0.57	0.26		Male	0.145	0.140	0.052	
	Intensity 20	Female	10.51	0.62	0.33	0.744	Female	0.093	0.085	0.042	0.785
		Male	10.42	0.84	0.39		Male	0.097	0.090	0.043	
4000 Hz	Intensity 60	Female	7.72	0.42	0.18	0.772	Female	0.164	0.155	0.062	0.340
		Male	7.77	0.42	0.26		Male	0.143	0.125	0.044	
	Intensity 40	Female	9.08	0.80	0.33	0.779	Female	0.125	0.110	0.061	0.911
		Male	9.01	0.55	0.34		Male	0.127	0.110	0.047	
	Intensity 20	Female	10.66	1.17	0.49	0.657	Female	0.131	0.075	0.178	0.327
		Male	10.47	1.00	0.62		Male	0.074	0.075	0.023	

Statistical test: Student's *t*-test; *p-value with a statistically significant difference
Subtitle: n = number of subjects; SF = specific frequency; CI = confidence interval

For the specific-frequency tone-burst stimulus, a statistically significant difference between the genders was observed. Such difference was verified for 4000 Hz frequency, in the intensities of 40 and 20 dBnHL for the latency values, and 60 and 40 dBnHL for the amplitude values. For the 500, 1000 and 2000 Hz frequencies, no differences were noted between the genders (Table 3).

In the analysis of the BAEP V-wave absolute latency values with the specific-frequency Ichirp stimulus, a statistically significant difference was verified between the genders only for the 1000 Hz frequency, in the intensity of 20 dBnHL, demonstrating higher mean values for females. In the analysis of the amplitude values, no statistically significant difference data were found (Table 4).

DISCUSSION

Through the results presented in this research, it was noted that the click and tone-burst stimuli were the ones with greater differences in the comparison analysis between the genders and that the broadband Ichirp was the stimulus that did not suffer influence for the analysis in question.

In BAEP recording with the click stimulus, a statistically significant difference was observed in the intensity of 60 and 40 dBnHL for the latency values, demonstrating lower mean values for females (Table 1). In the amplitude analysis, higher values were verified for females, with a statistically significant difference in 20 dBnHL. Such results agree with a study[26] in which the authors also found shorter V-wave latency values in 72 full-term female newborns, with the click stimulus. However, such a difference was not statistically significant. One justification for that result is in researchers'[29] reporting a shorter sound wave traveling time in the basilar membrane of females, which would mean a shorter time to generate a response in the brainstem; consequently, there would be a shorter wave latency, as well as a greater amplitude, as they generate more neural activity per unit of time. These data are attributed to the anatomical differences there are between the genders – e.g., the size of the cochlea and the diameter of the auditory nerve[30]. In addition to the anatomical differences, a study observed that the females can present better hearing in high frequencies[5], which would reflect in lower latencies, particularly for the click stimulus, due to the frequency range assessed. Another factor that could interfere with the responses in this potential is the female's higher mean body temperature[5]. Regarding the higher amplitude values, a study associated this result with the influence of hormone and neurotransmitter variations[5].

On the other hand, for the broadband Ichirp stimulus, this difference was not statistically significant (Table 2). Thus, it is understood that this stimulus presents relevant clinical applicability, given that, for the audiological diagnosis in children, stimuli are sought that are not influenced by gender. Attention is called to the fact that no studies were found in the literature consulted that had performed such comparison, using the Ichirp stimulus. However, neither did the researchers find any difference between genders when using chirp-class stimuli – e.g., A-chirp[21] and CE-chirp[10]. It should be emphasized that the chirp stimulus' characteristics are different from those of other stimuli, as it is projected to stimulate the cochlea simultaneously, enabling greater neural synchrony to take place and thus generate more reliable responses, when compared with the other stimuli[17]. Therefore, it is less likely to be influenced by anatomical aspects found between the genders. Furthermore, studies that analyzed and compared BAEP responses with the chirp and click stimuli in newborns had promising results with the chirp, as they observed that the V-wave was better identified in the BAEP recordings when using a chirp-class stimulus in newborns[11,14].

In the analysis of the tone-burst stimulus (Table 3), statistically significant latency difference was obtained for 4000 Hz frequency, in the intensities of 40 and 20 dBnHL. For the amplitude, a difference was verified for 4000 Hz frequency in the intensities of 60 and 40 dBnHL, with higher values for females. Other researchers[27] reported that they did not find a statistically significant difference between genders with the tone-burst stimulus. However, the study that did observe a difference between the genders[24] stated that such a difference was at random.

In this study, when analyzing the specific-frequency Ichirp stimulus data (Table 4), 12 statistical analyses were conducted, in which a statistically significant latency difference was found in one single frequency and intensity (20 dBnHL in 1000 Hz). Thus, it is inferred that this difference does not have clinical relevance either, so there is no need to report in the literature gender-related reference values. Neither were there any relevant differences between genders found in another study[28] with the SF CE-chirp stimulus when comparing the V-wave responses of 168 newborns.

Since the specific-frequency Ichirp stimulus presented statistical difference in only one frequency and intensity, when the BAEP recorded values for both males and females were compared, this study highlights that this is the stimulus indicated to investigate electrophysiological threshold in the newborn population. Moreover, the classic literature[17,19] points out that the chirp stimulus presents greater amplitudes, as the hair cells depolarize more synchronically, which enables more nerve fibers to be simultaneously activated. These researchers highlighted that, with the use of the chirp stimulus, when the level of stimulation decreases, the response amplitude remains stable.

CONCLUSION

The BAEP V-wave in newborns is influenced by gender when the click and tone-burst stimuli are used. However, such influence did not occur when the broadband (BB) Ichirp and specific-frequency Ichirp stimuli were used.

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