Does the Number of Stimuli Influence the Formation of the Endogenous Components of the Event-Related Auditory Evoked Potentials?

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Abstract

Introduction The number of stimuli is important to determine the quality of auditory evoked potential records. However, there is no consensus on that number in studies, especially in the sample studied.

Objectives To investigate the influence of the number of rare stimuli on forming N2 and P3 components, with different types of acoustic stimuli.

Methods Cross-sectional, descriptive, comparative study, approved by the ethics committee of the institution. The sample comprised 20 normal hearing adults of both sexes, aged 18 to 29 years old, with normal scores in the mental state examination and auditory processing skills. The event-related auditory evoked potentials were performed with nonverbal (1 kHz versus 2 kHz) and verbal stimuli (/BA/ versus /DA/). The number of rare stimuli varied randomly in the recordings, with 10, 20, 30, 40, and 50 presentations.

Results P3 latency was significantly higher for nonverbal stimuli with 50 rare stimuli. N2 latency did not show any difference between the type and number of stimuli. The absolute P3 and N2-P3 amplitudes showed significant differences for both types of stimuli, with higher amplitude for 10 rare stimuli, in contrast with the other ones. The linear tendency test indicated significance only for the amplitude – as the number of rare stimuli increased, the amplitude tended to decrease.

Conclusion The components were identifiable in the different numbers of rare stimuli and types of stimuli. The P3 and N2-P3 latency and amplitude increased with fewer verbal and nonverbal stimuli. Recording protocols must consider the number of rare stimuli.

Keywords
- evoked potentials
- P300 component
- hearing
- electrophysiology
- auditory cortex
- healthy volunteers

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Introduction

The auditory system, divided into the peripheral and central parts, has been widely studied to ensure that its measurements are as effective as possible.[1] One way to assess the central auditory nervous system is with the auditory evoked potentials, which in turn are classified according to their latency – that is, the time in milliseconds (ms) between the acoustic input and the formation of the target component throughout the central auditory pathway. Thus, they are divided into short-, middle-, and long-latency auditory evoked potentials.[1,2]

The event-related auditory evoked potentials (ERAEP) are long-latency evoked potentials (LLAEP) whose components are N2 and P3. Its recording is observed with the oddball paradigm, which consists of the presentation of at least two stimuli with some different tracing between them (such as sound frequency, intensity, and duration).[2,3] The person being assessed is told to ignore the frequent sounds and pay attention to the number of times the rare sounds occur. Thus, ERAEP is influenced by factors supramodal to hearing, such as memory and attention,[2,4,5] and are called endogenous components, which reflect the activity in the auditory pathway between the thalamus and cortex.[2,3,6]

The stimulus and recording parameters chosen to obtain the ERAEP are important because they influence the tracing, making the investigation of the cortical auditory pathways more reliable.[6] The type of stimuli (verbal or nonverbal), the total number of presentations, the proportion between frequent and rare stimuli, the intrastimulus interval, and their duration and intensity stand out among the stimulation parameters. All parameters must be chosen carefully because they directly influence the quality of the N2 and P3 components.[2,6,7]

The verbal stimuli (also called speech stimuli) and nonverbal stimuli (also called pure-tone stimuli) are used in ERAEP protocols, either alone or in combination, to compare the electrophysiological cortical auditory processing.[5,6] Verbal and nonverbal stimuli processing are known to have contrasting acoustic and neurophysiological properties in the auditory cortex, with hierarchical auditory organization and left-sided responses to the speech sounds, besides the high sensitivity to verbal sounds in the left anterior upper temporal cortex.[8] Unlike the nonverbal sounds, using speech sounds provides additional information on the processing of complex signals and of the speech signal when behavioral assessments are imprecise.[8–10] Therefore, choosing the type of acoustic stimuli is another important factor when deciding the protocol that will be used.[10]

Since ERAEP are triggered by the attention to the rare sound to the detriment of the frequent one, the proportion between them is crucial[7,11]–the most usual rates are, respectively, 20 and 80%.[2,6] Another aspect to consider is the habituation phenomenon, even at 20%. If there are many rare stimuli, the recording of the components is attenuated because it comes to be noticed as nonrare.[12] The total number of stimuli in the ERAEP survey is heterogeneous. However, there is a clear consensus in the literature regarding the distribution of rare and frequent stimuli.[1,7]

The total number of stimuli for ERAEP (that is, the mean number of stimuli), even in the recommended proportion, influences the duration of the examination – which can make the survey of this potential unfeasible in populations of particular interest.[13–15]

The present study was conducted based on clinical assumptions and specific conditions. Choosing a reliable number of rare stimuli is difficult, and it must consider the attention span of the subjects to the target stimuli and the complexity of the type of stimuli.[2,7,15] The objective of the present study was to measure N2 and P3 latency and amplitude obtained with different verbal and nonverbal sounds while keeping a fixed proportion between rare and frequent stimuli. The present research addressed the importance of the number of rare stimuli and the duration of this auditory evoked potential to the quality of the exam regarding changes in behavioral and neurodevelopmental disorders.

Methods

This is a cross-sectional, descriptive, comparative study, with a deductive research approach, focused on the diagnosis. It was performed at the Specialized Otorhinolaryngology and Speech-Language-Hearing Center of the Hospital das Clínicas of the Faculdade de Medicina of Ribeirão Preto.

Sample

The study was approved by the Research Ethics Committee of the originating institution (CAAE 05071718.7.0000.5440), comprising 20 subjects of both sexes aged 18 years to 29 years and 11 months old. They were recruited by invitations posted in the said institutions and/or digital media. The participants signed an informed consent form.

Procedures

The initial procedures, called prestudy, included an interview, assessments, and/or screening, whose abnormal results could lead to a study bias. Hence, they were considered exclusion tools for ERAEP assessment.

They were interviewed to identify neuropsychiatric signs and symptoms or cognitive complaints, medication use acting on the central nervous system, and alcohol, licit and illicit drug use 24 hours before the examination. All subjects had normal hearing, assessed with pure-tone and speech threshold audiometry (Madsen Astera,[2] Otometrics – Clinical Audiometer) and acoustic immittance measures (Madsen Zodiac 901, Otometrics – probe tone 226 Hz at 85 dB SPL). Normal hearing of the patients was classified based on mean dB HL thresholds at 0.5, 1, 2, and 4 kHz, according to 2104 World Health Organization criteria – mean thresholds $\leq$ 25 dB HL indicated normal hearing. The speech recognition threshold (SRT), with trisyllabic Portuguese words, was used to confirm the veracity of the thresholds; results $\leq$ 10 dB SL in the 3-frequency mean (0.5, 1, and 2 kHz) were interpreted as adequate. Acoustic immittance measures were considered adequate with types "A," "As," or "Ad" tympanograms and contralateral acoustic reflex present at 0.5 to 2 kHz.

The Mini-Mental State Examination, second edition (MMSE-2)[16] was used for cognitive screening. Adequate results were based on the cutoff score for 18- to 29-year-old subjects who attended school for 9 to 12 years, which is $\geq$ 25 points out of the 30 in the total score. For those who attended school for $\geq$ 13 years, the cutoff is 27 points ($T < 36$ score).

Behavioral Auditory Processing Tests

Temporal ordering and resolution skills were respectively investigated with the Frequency Pattern Test (FPT)[17] and Random Gap Detection Test (RGDT).[18] The FPT was conducted with 30 sequences presented diotically at 50 dBSL. The subjects were instructed to name the three sounds in the order they heard them based on their frequency (low versus high). A percentage $\geq$ 76% of correct answers was considered normal.[19] The RGDT was performed diotically at 50 dBSL at 0.5, 1, 2, and 4 kHz, plus the training track, each one with 10 presentations and intervals of 0 to 20 milliseconds. The subjects were instructed to identify whether they heard one or two stimuli in each presentation. Adequate results were mean temporal thresholds $\leq$ 10 milliseconds at the 4 frequencies.[18] The dichotic verbal listening mechanism was assessed with the Dichotic Digits Test,[20] binaural integration stage, at 50 dBSL. The subjects were instructed to repeat the four numbers they heard in each sequence, regardless of their order. The adequate result was $\geq$ 95% of correct answers.

Event-related auditory evoked potentials

Event-related auditory evoked potentials was conducted on all subjects who had adequate results in the aforementioned procedures. The 2-channel equipment used for this auditory evoked potential was manufactured by Intelligent Hearing Systems (IHS), module SmartEP, with insert earphones model ER3A. After cleaning their skin, surface electrodes were fixed according to the International 10–20 System, as follows: negative electrodes at A1 (left earlobe) and A2 (right earlobe), positive electrodes at Cz (vertex), and the ground electrode at Fpz (forehead). The impedance level was maintained between 1 and 3 kΩ.

Two sets of stimuli were used, namely: nonverbal (tone-burst stimuli) – frequent stimuli at 1,000 Hz and rare stimuli at 2,000 Hz; and verbal (speech stimuli) – syllable /BA/ as frequent and /DA/ as rare stimuli. Both the 1 and 2 kHz stimuli lasted 10,000 milliseconds. The speech stimuli, according to information from IHS (SmartEP), had the following characteristics: /BA/ lasted 114.87 milliseconds (18 milliseconds for the consonant and 75 milliseconds for the vowel); pitch (beginning – end) at 112.4 Hz and 1,112 Hz; first formant (F1) at 818 Hz, second (F2) at 1,378 Hz, third (F3) at 2,024 Hz, fourth (F4) at 2,800 Hz, and fifth formant (F5) at 4,436 Hz. The characteristics of the nonfrequent syllable /DA/ were as follows: duration of 206.27 milliseconds (9 milliseconds for the consonant and 174 milliseconds for the vowel); pitch (beginning – end) at 109.1 Hz and 102.1 Hz; first formant (F1) at 732 Hz, second (F2) at 1,335 Hz, third (F3) at 2,498 Hz, fourth (F4) at 3,058 Hz, and fifth formant (F5) at 3,828 Hz. Both stimuli were presented at 75 dBnHL. The analysis window was from - 25 to 512 milliseconds, with a 50,000 gain, at a stimulus presentation rate of 1.1 per second, with alternating polarity and a 1 to 30 Hz filter.

The rare stimuli were randomly presented, randomized by the software, in the proportion of 20% rare to 80% frequent stimuli. With the oddball paradigm, the subjects were expected to identify the rare stimuli from the frequent ones in the presentation. The test comprised 5 trials, with 10, 20, 30, 40, and 50 rare stimuli respectively in trials 1, 2, 3, 4, and 5, randomly applied to each subject and using the two stimuli. Subjects were in the horizontal position in a dim room and were instructed to remain attentive and relaxed and mentally count the rare stimuli. To validate the record and ensure the subjects remained attentive to the rare stimuli, ± 2 was accepted as a response. The total trial time was $\sim$ 2 minutes (m) for 10 rare stimuli; 4 m for 20; 6 m for 30; 8 m for 40; and 10 m for 50 rare stimuli.

All the aforementioned stages were conducted on a single day.

Long-latency evoked potentials performed with the other stimuli (tone-burst or speech, depending on the randomization) was scheduled for another day, so the subject would return for data collection at an interval of 7 to 10 days. This care was necessary for LLAEP not to present fatigue response in the recordings. Long-latency evoked potentials with the second type of stimuli was performed in the same application conditions. In both LLAEP applications, between the 2 recordings, the evaluator interacted with the subject to provide an interval of 5 to 10 minutes.

N2 and P3 components were analyzed considering the wave recorded with the rare stimuli. The latency was identified based on the Hall III criteria[2]–that is, from 180 to 250 ms for N2 (second negative-going wave) and from 200 to 380 ms for P3 (third positive-going wave). The absolute amplitude of each peak was analyzed, as well as the peak-to-peak value (N2-P3).

Statistical Analysis

The inferential data analysis was made with IBM SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). The repeated measures analysis of variance (ANOVA) was used for N2 and P3 latency and amplitude measures. Bonferroni post hoc test was applied when there was a significant difference. The statistical verification of measures with a linear trend throughout the stimuli was applied for latency and amplitude. The statistical significance level was set at $p \leq 0.05$.

Results

A total of 20 subjects accepted the invitation and attended the assessments – all of them had adequate results in prestudy procedures. Of these, 55% ($n = 11$) were females and 45% ($n = 9$) were males. Their age ranged from 18 years to 29 years and 5 months old, with a mean of 21.45 years ($\pm$2.87, and a standard deviation [SD] of 0.64).

Table 1 Description, in absolute numbers and percentages, of the recorded endogenous components of the subjects that comprised the sample

Component	Type of stimulus	Number of rare stimuli (trials)				
		10	20	30	40	50
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
N2	Pure tone 1 and 2 kHz	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
	Speech /BA/ and /DA/	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)
P3	Pure tone 1 and 2 kHz	19 (95%)	20 (100%)	19 (95%)	19 (95%)	20 (100%)
	Speech /BA/ and /DA/	20 (100%)	19 (95%)	19 (95%)	18 (90%)	19 (95%)

Abbreviations: *n*, absolute number; %, percentage.

In the initial analysis, component identification was studied in relation to the various trials (►**Table 1**). N2 was observed in 100.0% of the subjects for the 2 types of stimuli and in the 5 trials. P3 was identified in 18 to 20 subjects, depending on the trial and type of stimuli. Although the difference in occurrence between the 2 components was only 1 to 2 subjects, nonverbal stimuli were identified in 100% of the subjects in trials 2 and 5, whereas only in trial 1 of the verbal stimuli the P3 was identified in 100% of the sample.

Latency

No significant differences were found in N2 latency values (►**Table 2**) for the two types of stimuli in relation to the various trials (nonverbal, $F_{4;76} = 0.78$; $p = 0.54$; verbal, $F_{4;76} = 1.00$; $p = 0.42$). Neither was a linear tendency observed (►**Fig. 1**) in the latency values for the same variables (nonverbal, $F_{1;19} = 0.06$; $p = 0.80$; verbal $F_{1;19} = 1.46$; $p = 0.24$).

There was a difference in P3 latency (►**Table 2**) for nonverbal stimuli between the 5 trials ($F_{4;72} = 3.01$; $p = 0.02$). The post hoc test showed that trial 5 resulted in higher values than trials 3 and 4 (respectively, $p = 0.04$ and $p = 0.002$). The linear analysis (►**Fig. 1**) showed no significant difference ($F_{1;18} = 3.76$; $p = 0.07$). P3 latency values for verbal stimuli showed no significant difference ($F_{4;68} = 2.29$; $p = 0.07$), though a tendency was observed (►**Fig. 1**). Therefore, the post hoc was performed, which showed higher values in trial 1 than in trial 2 ($p = 0.04$). However, there was no response with linear tendency ($F_{1;17} = 0.03$; $p = 0.86$; ►**Fig. 1**).

Amplitude

N2 amplitude values (►**Table 3**) for nonverbal stimuli were not statistically different in the various trials ($F_{4;76} = 2.39$; $p = 0.06$); in fact, they were quite close. Therefore, the post hoc was performed, which showed higher values in trial 2 than in trial 3 ($p = 0.05$). The linear analysis (►**Fig. 1**) revealed a decrease in amplitude values as the trials increased in number (1, 2, 3, 4, and 5) ($F_{1;19}$= 4,40; $p = 0.05$). No significant difference in N2 amplitude was found for verbal stimuli in the various trials ($F_{4;76} = 1.96$; $p = 0.11$), neither was there a linear tendency ($F_{1;19} = 0.06$; $p = 0.80$; ►**Fig. 1**).

A significant difference was verified for P3 (►**Table 3**) obtained with nonverbal stimuli ($F_{4;72} = 6.42$; $p = 0.001$). The post hoc test showed higher values in trial 1 than in trial 3 ($p = 0.05$) and 4 ($p = 0.01$). A linear tendency was also observed in this analysis ($F_{1,19} = 10.39$; $p = 0.005$; ►**Fig. 1**) – there was a tendency to decrease in amplitude as the trials increased in number. A significant difference was verified in P3 amplitude for verbal stimuli between the various trials ($F_{4;68} = 4.94$; $p = 0.001$). The post hoc test showed that the amplitude in trial 1 was higher than in trial 4 ($p = 0.03$). A linear tendency was also observed ($F_{1;17} = 11.47$; $p = 0.003$; ►**Fig. 1**) – the amplitude tended to be significantly lower as the trials increased in number.

Peak-to-peak (N2-P3) amplitude values for nonverbal stimuli were significantly different ($F_{4;72} = 7.30$; $p < 0.001$). The post hoc test showed that N2-P3 amplitude in trial 1 was significantly higher than in the other trials – trial 1 versus 2 ($p = 0.04$), trial 1 versus 3 ($p = 0.02$), trial 1 versus 4 ($p = 0.008$), and trial 1 versus 5 ($p = 0.4$). A significant difference was observed for verbal stimuli ($F_{4;68} = 6.01$; $p < 0.001$). The post hoc analysis showed that trial 1 had higher amplitudes than trials 3 and 4 ($p = 0.003$ and $p = 0.008$, respectively). There was a linear tendency in the data ($F_{1;18} = 13.04$; $p = 0.002$; ►**Fig. 1**), showing a decrease in amplitude as the trials gradually increased in number. In linear analysis (►**Fig. 1**), the results revealed a significant linear tendency ($F_{1;17} = 10.17$; $p = 0.005$) – the amplitude tended to be lower as trials increased in number.

The mean of all records can be verified below, encompassing both stimuli, in the variables analyzed (►**Fig. 2**).

Discussion

Various factors that influence the quality of ERAEP recordings have been reported in the literature,[2,5,6] including the stimulation parameters.[2,3] N2 and P3 endogenous component responses are related to processes supramodal to hearing, especially attention.[2] The proportion between the frequent and distracting stimuli is essential to trigger the components and ensure greater reliability of recordings, as it has been discussed since the 1970s.[1,7,21,22]

Table 2 N2 and P3 mean latencies and standard deviation for pure-tone and speech stimuli in the five different numbers of rare stimuli mutually compared ($n = 20$)

Type of stimulus	Component	Number of rare stimuli	Mean (ms)	SD (ms)	Test[a] p-value	Post-test[b] p-value
Pure tone 1 and 2 kHz	N2	10	287.80	39.72	0.54	NA
		20	295.30	27.43		
		30	293.60	37.46		
		40	283.60	35.72		
		50	291.35	32.39		
	P3	10	379.89	37.84	0.02*	0.04 50 > 30 0.002 50 > 40
		20	387.45	33.94		
		30	380.89	19.81		
		40	380.26	28.10		
		50	399.45	26.30		
Speech /BA/ and /DA/	N2	10	285.75	37.39	0.42	NA
		20	272.55	33.04		
		30	274.50	28.73		
		40	260.72	65.59		
		50	273.70	34.56		
	P3	10	389.75	34.77	0.07	NA
		20	363.57	37.96		
		30	377.68	35.01		
		40	381.27	39.00		
		50	382.15	38.03		

Abbreviations: ms, milliseconds; NA, not applicable; SD, standard deviation.
[a] ANOVA.
[b] Bonferroni post hoc test.

The literature that addresses ERAEP parameters reports that ~ 20 to 50 rare stimuli are necessary to clearly produce endogenous components – as long as these absolute values correspond to 20% of all stimuli.[1,7,21] Accordingly, the result of the present study is corroborated by this literature, as the five trials furnished the identification of both components, with no difference between them.

Concerning latency, the results in the present study pointed out that, for P3 with nonverbal stimuli, trial 5 (50 rare stimuli out of the total 250) resulted in higher latencies than trials 3 (30 rare stimuli out of the total 150) and 4 (40 rare stimuli out of the total 200). Hence, when using trial 5, prolonged P3 latencies are to be expected – which requires careful comparison with other studies using different mean parameters. Authors have long reported that the rarer the stimuli for ERAEP, the greater the latency, amplitude, and morphology variations in the records and their components.[7,21]

The influence of the various trials on P3 amplitude was observed in the two types of stimuli. The increase in mean numbers, and consequently in rare stimuli, attenuated the values of this variable. This result is backed by the literature, which reports that the less likely the occurrence of the rare stimuli, the higher the component amplitude, because the person has the attention sustained in a sequence of frequent stimuli.[7,21,22]

The various trials did not result in different N2 amplitudes. This result was not expected, although an observation must be made.

The higher P3 and N2-P3 amplitude values were obtained in trial 1 (10 rare stimuli out of the total 50) – that is, the trial with the shortest testing time. Since ERAEP components are modulated by attention,[2] it can be hypothesized that the shorter testing time favored sustained attention, reflecting on a more robust recording.

Cortical electrophysiological activity, which is involved in the recording of ERAEP endogenous components, is directly related to attention, discrimination, memory, integration, and decision-making skills.[2,3,11] These components that originate in the region of the thalamus, the auditory cortex, and cortical association areas involve structures that act upon tasks that require detection, acoustic stimulus discrimination and sensation, auditory memory, integration, auditory attention, and alert state.[2,4,23] Thus, attention and recent working memory depend on the discrimination between the stimuli, whether verbal or nonverbal.[4,24,25]

Beyond this finding, some conditions that limit the cognitive and behavioral aspects (whose characteristic is the

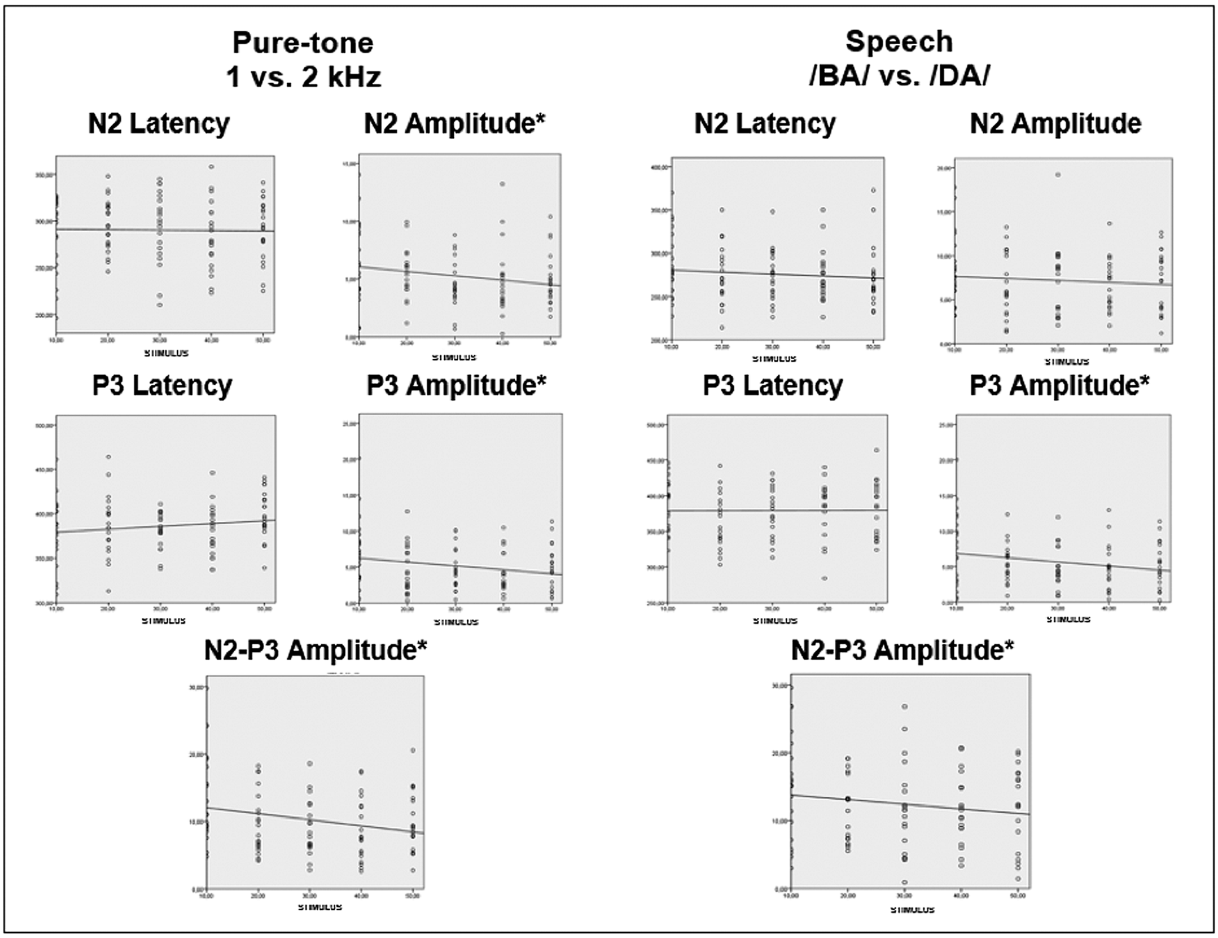

Fig. 1 Linear tendency of variation of the N2 and P3 latency and amplitude values (absolute and peak-to-peak) for both acoustic stimuli in the different numbers of rare stimuli. *= statistically significant.

difficulty regulating the sustained attention to activities) diverge from subjects with good cognitive-behavioral performance – for example, intellectual disability and attention-deficit/hyperactivity disorder.[26,27] These populations lead us to consider adaptations in LLAEP recording protocols, such as the number of rare stimuli, since they have a shorter attention span.[15,26] The MMSE-2, administered for cognitive screening in subjects of the present research, encompasses aspects related to orientation, memory, attention, and specific skills, such as naming and comprehension. However, it does not substitute a comprehensive assessment because it is not sensitive enough to detect subtle cognitive variations.[16]

The type of stimuli is one of the factors that directly influence the recording of LLAEP endogenous components. Acoustic temporal complexity of the stimuli used (verbal and nonverbal) activates different cortical regions and presents a distinct functional organization in the human auditory cortex.[2,8,9] In the present study, the latency and amplitude values were slightly higher for speech stimuli. The literature points out that, when speech stimuli are used to record LLAEP endogenous and exogenous components, they influence their latency and amplitude measures. This occurs because the complexity of speech stimuli transcends the less hermetic processing of pure tones.[9] Moreover, studies indicate that electrophysiological recordings using speech sounds (synthetic syllables) provide complementary information to that obtained with standard behavioral assessments, either due to the auditory processing of speech or to aspects supramodal to hearing.[4,9,10] The speech stimuli /BA/ and /DA/, respectively the frequent and rare stimuli, differ in terms of duration and articulation point for identification, making them more complex and affecting the speed and quality of the auditory processing.[10,25]

Some limitations can be pointed out in the present study, such as the small sample size, since more subjects could make the tendency values (which drew near to significance) more robust. Also, a battery of neuropsychological assessments could be used to ensure more reliable control of attention, memory, and cognition. Nevertheless, future studies should be attentive to the protocols established to record LLAEP endogenous components, especially regarding the number of stimuli and the sample to be studied, always considering their conditions concerning sustained attention and working memory.

Conclusion

In conclusion, with the parameters approached in the present study, it was identified that the various trials

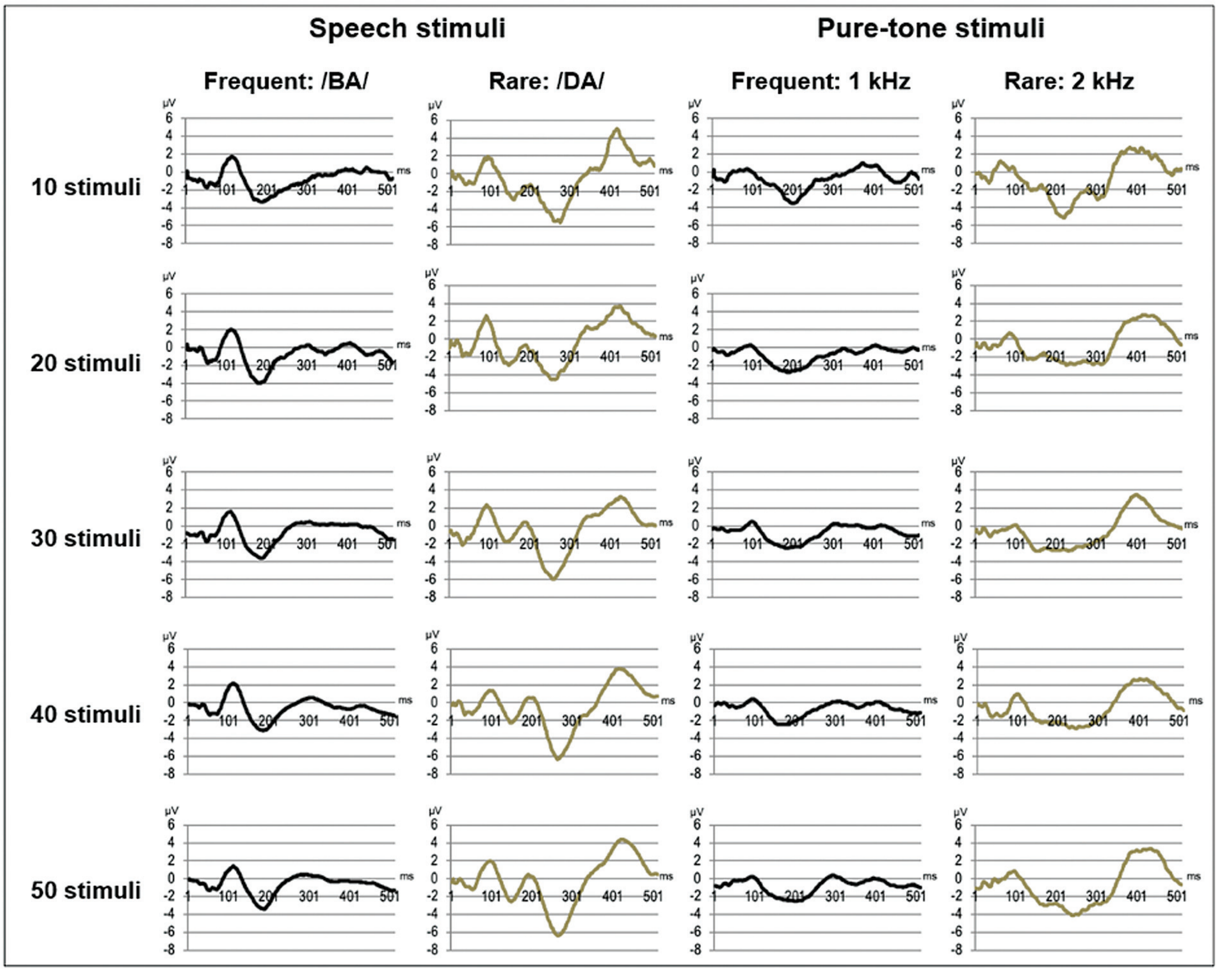

Fig. 2 Representation of the ERAEP mean records from the 20 subjects, according to the different numbers of rare stimuli and types of stimuli.

Table 3 N2 and P3 components and N2-P3 peak-to-peak mean amplitudes and standard deviation for pure-tone and speech stimuli in the five different numbers of rare stimuli mutually compared ($n = 20$)

Type of stimuli	Component	Number of rare stimuli	Mean (μV)	SD (μV)	Test[a] *p*-value	Post-test[b] *p*-value
Pure tone 1 and 2 kHz	N2	10	6.53	3.42	0.06	NA
		20	5.47	2.04		
		30	4.60	2.04		
		40	4.82	2.89		
		50	4.95	2.19		
	P3	10	7.35	4.55	$< 0.001^*$	0.05^* $10 > 30$ 0.01^* $10 > 40$
		20	4.67	3.26		
		30	4.72	2.87		
		40	4.18	2.64		
		50	4.81	3.03		
	N2-P3	10	13.92	6.78	$< 0.001^*$	0.04^* $10 > 20$ 0.02^* $10 > 30$ 0.008^* $10 > 40$ 0.04^* $10 > 50$
		20	9.78	4.43		
		30	8.94	4.05		
		40	8.71	4.57		
		50	9.82	4.30		

Table 3 (*Continued*)

Type of stimuli	Component	Number of rare stimuli	Mean (μV)	SD (μV)	Test[a] *p*-value	Post-test[b] *p*-value
Speech /BA/ and /DA/	N2	10	8.39	3.96	0.11	NA
		20	6.42	3.61		
		30	7.27	4.00		
		40	6.73	2.85		
		50	7.12	3.37		
	P3	10	7.54	5.28	0.001*	0.03* 10 > 40
		20	5.50	2.65		
		30	4.94	2.82		
		40	4.81	3.24		
		50	5.24	3.04		
	N2-P3	10	15.06	7.60	< 0.001*	0.003* 10 > 30 0.008* 10 > 40
		20	11.79	4.59		
		30	11.63	6.72		
		40	11.65	5.05		
		50	11.79	5.84		

Abbreviations: NA, not applicable; SD, standard deviation; μV, microvolts.
[a] ANOVA.
[b] Bonferroni post hoc test.

with different means influenced N2 and P3 formation. Amplitude was more influenced than latency with both verbal and nonverbal stimuli. The amplitude values also tended to decrease as the mean number of stimuli increased. Only P3 with nonverbal stimuli had a prolonged latency with the mean of 250 presentations (50 rare stimuli), in contrast with the other rates. The number of rare stimuli used in research and clinical protocols must be considered, being always attentive to the different conditions of the target population in whom LLAEP will be conducted.

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Conflict of Interests
The authors have no conflict of interests to declare.

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