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ABSTRACT

Purpose: To analyze the latency and amplitude of P300 responses obtained with electrodes positioned at Cz and Fz and in different tasks of infrequent stimulus identification in normal hearing individuals. **Methods:** Forty adults of both genders participated in the study. Three recordings with three different tasks were obtained for the identification of the infrequent stimulus; simultaneous recordings were obtained from Cz and Fz positions. **Results:** Cz position showed significantly greater amplitudes and lower latencies than Fz. Regarding the three tasks, only “pronouncing the word ‘thin’” was different from “raising a finger”, with lowest latencies for the verbal task. Regarding amplitude, significantly higher values were observed for “raising a finger”, followed by “pronouncing the word ‘thin’” and mental counting. **Conclusion:** Cz obtained the best values, highest amplitude and lowest latency. Lowest latencies were obtained for the task of “pronouncing the word ‘thin’” and the highest amplitudes were obtained for “raising a finger”.

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INTRODUCTION

There has been a significant increase in publications focused on studying the Hearing System, mainly regarding the Central Auditory Nervous System (CANS), by behavioral, electrophysiological and imaging systems⁽¹⁾.

One form of CANS assessment is through evoked potentials obtained by recording and measuring responses to sensory stimuli captured on the skull surface. Long-latency evoked potentials, especially cognitive potentials (CP), are directly influenced by the patient motivation, his/her level of attention, the type of task requested and stimulus of previous experience. P300 CP is also denoted endogenous or event-related potential^(2,3).

Hearing assessment using long-latency potentials in combination with behavioral tests to the central hearing system has proved to be a promising and potent method to the best understanding of this system⁽⁴⁾.

Among different audiological procedures that assess the central auditory system, integrity is the information obtained through auditory evoked potentials. These, in turn, play a significant role in audiology, since they provide data on nervous system in response to acoustic stimulation, are non-invasive and provide information to monitor the natural speech and hearing development when necessary⁽⁵⁾.

The Long-Latency Auditory Evoked Potential (LLAEP), P300, has been studied to assess hearing function in different situations. It can also be a clinical accessory to the neurophysiological measurement of the cognitive process.

The motivation to establish a protocol for behavioral assessment and individuals follow-up with variation in the hearing system in combination with objective assessments has increasingly been emphasized in the literature, not only for individuals with hearing loss or attention disorders, but also for individuals with degenerative diseases, allowing a data comparison during the disease course, both in terms of speech, writing and auditory perception⁽⁶⁾.

Few studies have reported normal P300 values for different age ranges compared to other diagnostic procedures, mainly regarding the parameters used for assessment and electrode positioning. A normative P300 study⁽⁷⁾ to establish latency and amplitude values for healthy young adults has reported wide variation.

There is no scientific evidence on the variability of latency measurements and P300 amplitude depending on the task given to the patient, and the best position of the active electrode.

Thus, the present study aimed to analyze latency and amplitude responses of P300 CP in normal individuals of both genders, in different positions for Fz and Cz electrodes and in different tasks of infrequent stimulus identification.

METHODS

Cross-sectional comparative study with 40 healthy volunteers (20 males and 20 females) aged 18 to 30 years (mean age: 22 years old) with normal hearing and with no signs of neuropsychological problems. The study was approved by the

Institutional Research Ethics Committee (number 12790/2011) and all individuals signed an informed consent.

Some variables were controlled (e.g. drug use, physical activity, and women hormonal cycle) in view of their influences on measurements of P300 latency and amplitude. The sensitive auditory was determined with pure-tone threshold audiometry (250 to 8000 Hz), speech audiometry thresholds, tympanometry and determination of ipsilateral and contralateral acoustic reflex at frequencies of 500, 1000, 2000 and 4000 Hz. Individuals with thresholds of more than 20 dBHL, type A tympanometry curve and presence of acoustic reflexes of stapedius muscle in the condition of contralateral afferent pathway were considered to have normal hearing.

The P300 test was performed using a Bio-Logic[®] apparatus, version 5.70 – model 317, tone burst stimulus, a 50.000 μ V gain, 75 dBnHL for infrequent (2000 Hz) and frequent stimuli (1000 Hz), presented in a binaural manner, a 1-30 Hz filter, a 512 ms window, and insertion phones as transducer. Impedance of electrodes was maintained at 3 k Ω or less. A total of 300 artifact-free stimuli were used (80% frequent stimuli and 20% infrequent stimuli). The presentation rate was 1.1 stimuli per second.

Electrodes were positioned as proposed by Jasper⁽⁸⁾, i.e., the active electrodes were positioned at Cz and Fz and were connected to entry 1 of the preamplifier of channels 1 and 2, respectively. Reference electrodes were placed on the earlobe (A1 and A2), interlinked and connected to entry 2 of channel 1 and interlinked to channel 2 by the jumper of the preamplifier. The ground electrode was placed at Fpz.

The subject was asked to identify the infrequent stimulus in three different tasks, i.e., 1) mental count (MC); 2) raising the right index finger (RI), and 3) pronouncing the word “thin” (PT), as illustrated in Figure 1. The order of the tasks was randomized.

The individuals were instructed to keep their eyes closed during the test in order to avoid the interference of the electrical activity of random or rapid eye movements such as blinking during the P300 recording.

The identification of infrequent stimuli, independently of the task, was controlled by the examiner, with replies containing the correct number of infrequent stimuli presented or with variations of more or less three numbers being accepted.

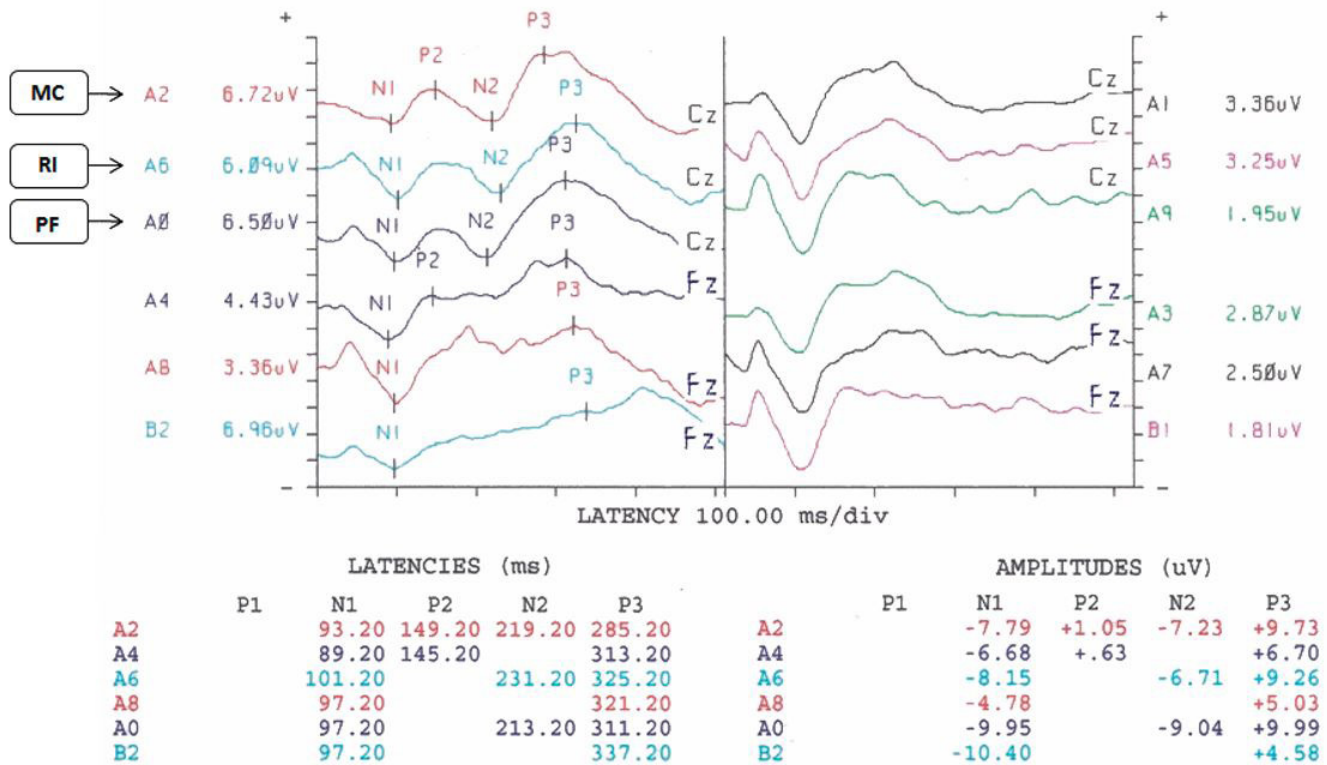
Data were tabulated and statistically analyzed with the SAS[®] 9.0 software using linear mixed-effects regression models (random and fixed effects), with the level of significance set at $p \leq 0.05$.

RESULTS

The descriptive analysis results (mean, standard and median deviation) of the P300 latency and amplitude measurements at the different position of electrode placement (Cz and Fz) in the three different tasks of infrequent stimulus identification (MC, RI and PT) and in the comparison of males and females in the same situation of P300 variability are presented in Tables 1 and 2.

Throughout the study, the latency and amplitude values of P300 (mean \pm SD) for the overall sample were 296.14 ± 15.95 ms and 5.72 ± 0.75 μ V, respectively. At the Cz and Fz positions, P300 latency and amplitude values were 282.70 ± 5.06 ms

B I O - L O G I C E V O K E D P O T E N T I A L R E P O R T



Caption: Cz = central position of the electrode at the central lobule; Fz = central position of the electrode at the frontal lobule; SD = standard deviation; MC = mental count; RI = raising the right index finger; PT = pronouncing the word "thin"; ms = milliseconds; μ V = microvolts

Figure 1. Example of P300 test performed in the present study, with markings in the three tasks of infrequent stimulus identification

Table 1. P300 mean, standard deviation and median latency and amplitude for males and females in the positions of electrode placement at Cz and Fz (N=40)

		P300			
		LATENCY (ms)		AMPLITUDE (μ V)	
		Cz	Fz	Cz	Fz
MALE (N=20)	Mean	286.29	306.86	5.85	4.91
	SD	39.61	41.26	2.9	2.94
	Median	284.2	316.2	5.53	4.2
FEMALE (N=20)	Mean	279.12	312.31	6.69	5.46
	SD	37.46	42.18	2.98	2.76
	Median	269.2	317.2	6.67	5.14

Caption: Cz = central position of the electrode at the central lobule; Fz = central position of the electrode at the c frontal lobule; SD = standard deviation; ms = milliseconds; μ V = microvolts

and 309.58 ± 3.85 ms for latency and 6.27 ± 0.59 μ V and 5.18 ± 0.38 μ V for amplitude, respectively.

When the results of latency and amplitude measurements were compared according to electrode positioning at Cz and Fz, we detected a significant difference for latency ($p = 0.0001$) and amplitude (<0.0001), with the electrode position on Cz showing the lowest latencies and highest amplitudes.

Comparison of male and female genders did not show a difference for latency ($p = 0.9313$) or amplitude ($p = 0.1960$) in the studied sample.

Analysis of latency measurements according to the three different tasks requested for identification of the infrequent stimulus showed a difference only between PT and RI tasks ($p = 0.0320$), with a lower latency for RI task, as shown in Table 3. When comparing the position of electrode placement according to the three different tasks for identification of the infrequent stimulus, we observed a difference for MC ($p = 0.0035$), RI ($p = 0.0008$) and PT ($p < 0.0001$), with a lower latency with the electrode in the Cz position, as seen in Table 4.

There was no difference in P300 latency for females in different tasks for identification of infrequent stimulus, whereas

Table 2. P300 mean, standard deviation and median latency and amplitude for males and females in the positions of electrode placement at Cz and Fz, according to the different tasks (N=40)

			P300			
			LATENCY (ms)		AMPLITUDE (µV)	
			Cz	Fz	Cz	Fz
MC	MALE	Mean	282.88	298.25	5.79	3.81
		SD	35.99	41.07	2.88	2.26
		Median	277.2	298.2	5.15	3.76
	FEMALE	Mean	286.85	310.26	6.76	4.73
		SD	38.68	46.66	2.82	1.43
		Median	274.2	320.2	7.14	4.88
RI	MALE	Mean	292.41	322.26	5.79	6.02
		SD	43.64	45.16	2.88	3.77
		Median	299.2	335.2	5.73	4.89
	FEMALE	Mean	274.65	323.48	6.99	7.14
		SD	38.43	42.07	3.41	3.75
		Median	262.2	334.2	6.26	5.75
PT	MALE	Mean	283.42	302.95	5.99	4.65
		SD	40.33	34.42	3.13	2.32
		Median	282.2	315.2	5.52	4.13
	FEMALE	Mean	276.73	304.66	6.33	4.46
		SD	36.16	36.74	2.8	1.92
		Median	269.2	309.2	6.39	4.53

Caption: Cz = central position of the electrode at the central lobule; Fz = central position of the electrode at the frontal lobule; SD = standard deviation; MC = mental count; RI = raising the right index finger; PT = pronouncing the word "thin"; ms = milliseconds; µV = microvolts

Table 3. Estimated difference and p value for P300 latency and amplitude measurements when comparing the three tasks for the identification of the infrequent stimulus (MC, RI and PT) (N=40)

	TASKS	ESTIMATE	P VALUE *
LATENCY	MC – PT	-6.9665	0.1226
	MC – RI	2.8799	0.5236
	PT – RI	9.8464	0.0320
AMPLITUDE	MC – PT	-1.1686	0.0022
	MC – RI	-0.00293	0.9938
	RI – PT	1.1657	0.0025

* p ≤ 0.05, statistically significant value

Caption: MC = mental counting; RI = raising the right index finger; PT = pronouncing the word 'thin'

Table 4. Estimated difference and p value for P300 latency measurements when the position of electrode placement was compared to the three different tasks for the identification of the infrequent stimulus (MC, RI and PT) (N=40)

TASK	ELECTRODE	ESTIMATE	P VALUE *
MC	Cz – Fz	-18.6099	0.0035
PT	Cz – Fz	-37.4724	<.0001
RI	Cz – Fz	-22.0861	0.0008

* p ≤ 0.05, statistically significant value

Caption: Cz = central position of the electrode at the central lobule; Fz = central position of the electrode at the frontal lobule; MC = mental counting; RI = raising the right index finger; PT = pronouncing the word 'thin'

a difference was detected between MC and PT (p = 0.0299) for the males.

Analysis of the amplitude values detected in the sample for the three different tasks for identification of infrequent stimulus revealed a difference between MC and PT (p = 0.0022) and between RI and PT (p = 0.0025). The highest amplitude values were observed for RI task, followed by PT and MC.

There was no difference in mean amplitude among the three different tasks for identification of infrequent stimulus in the males, i.e., MC and PT (p = 0.0724), MC and RI (p = 0.5488), and PT and RI (p = 0.2493). For females, there was a difference in mean amplitude between the three tasks, i.e., MC and PT (p= 0.0100) and PT and RI (pv= 0.0014). PT was the task for identification of infrequent stimulus that showed the greatest amplitude of P300, Table 5.

DISCUSSION

Factors that could interfere with the recording of endogenous potentials are often described in the literature, such as age, gender, help states, awareness, psychological conditions, circadian cycle, among others. The type of the task is another factor that has drawn attention from researchers about changes in amplitude measures and P300 latency recordings.

The average of latency measurements and amplitude of P300 for three infrequent stimulus identification tasks were 296.14 ms and 5.69 µV; values within the range given in the

Table 5. Estimated difference and p value for P300 latency and amplitude measurements when males and females were compared to the three different tasks for the identification of the infrequent stimulus (MC, RI and PT) (N=40)

	SEXES	TASKS	ESTIMATE	P VALUE *
LATENCY	F	MC – PT	0.09614	0.9878
		MC – RI	6.9206	0.2675
		PT – RI	6.8245	0.2755
	M	MC – PT	-14.0291	0.0299
		MC – RI	-1.1609	0.8589
		PT – RI	12.8683	0.0542
AMPLITUDE	F	MC – PT	-1.3695	0.0100
		MC – RI	0.3213	0.5379
		PT – RI	1.6908	0.0014
	M	MC – PT	-0.9677	0.0724
		MC – RI	-0.3272	0.5488
		PT – RI	0.6405	0.2493

* $p \leq 0.05$, statistically significant value

Caption: F = female sex; M = male sex; MC = mental counting; RI = raising the right index finger; PT = pronouncing the word 'thin'

literature for the reference values established for young adults and adults, 17-30 years^(9,10).

In this study, there was no significant difference when comparing genders with average latency and amplitude of P300 recordings. There is no consensus in the literature regarding the difference of latency and amplitude measures between genders. Investigations, with similar results to those found in this study^(11,12), also confirmed that there was no relationship between the variable gender with variations in latency and amplitude. However, Franco⁽²⁾ shows the difference between genders, with lowest average values and standard deviation for P300 latency for females. Possibly the issue of latency measures variability related to gender, regarding the hormonal cycle influence, will remain as objective of difficult monitoring.

When comparing the P300 results obtained when placing the electrodes at Cz and Fz, there is a difference between latency measurements ($p < 0.0001$) and amplitude ($p = 0.0001$), with better wave morphology when the electrode was positioned at Cz (Table 4).

It is recommended the placement as close as possible to the surface electrode presumed as neural generator⁽¹⁾. Recommendations observed in different studies are the placement of active electrodes at Cz and Fz or Cz, Fz and Pz or just Fz or Cz⁽¹²⁻¹⁵⁾. The variation in the positioning of anterior frontal portion and the posterior occipital portion decreases the amplitude of the waves, showing that the maximum amplitude is generally obtained at Fz/Cz or the electrode position Fz/Pz⁽⁴⁾.

The values of latency and amplitude, with the fixed electrodes at Cz and Fz, found in this research (Table 1), equated to values obtained by Franco⁽²⁾. The difference between the electrodes positions of Fz-Pz and Cz-Pz (Table 3), for latency, was also described by Duarte et al.⁽¹²⁾.

Thus, these results reinforce the best placement of the electrode for capturing the potential cognitive P300 in Cz position.

The type of task requested for P300 evaluation is another factor to be considered when performing the exam, since

attention is a condition required for the characteristic wave of the cognitive potential to be observed⁽¹⁶⁾. Many of the published papers failed to describe the specific information on the type of task requested for the participants to identify the infrequent stimulus. They reported the type of command, such as "pay attention to the infrequent stimulus," and "respond only to the infrequent stimulus"⁽¹⁷⁻¹⁹⁾. The types of tasks requested for the identification of the stimulus infrequent detected in the literature surveyed were: "mental counting"^(2,3,7,9,11,20,21); "raising the finger"⁽⁶⁾; "counting aloud"^(12,22); "pressing the button"^(23,24) and "counting and raising one hand"⁽²⁵⁾.

Although the task of "mentally counting" has been the most found in the literature, it is also the task that can cause more difficulties for the record analysis, because the patients with cognitive disorders or without evaluator effect control to the number reported at the end of the test can compromise the performance of the task or registration. Therefore, the performance relative to the task should be analyzed by the evaluator, in order to ensure that the measures of amplitude and latencies obtained during the registration reflect the proposition of the test. It seeks to understand the test answers variation based on the task to control the variable type of task in the clinical use of the P300, when it is used to help the assessment of the individual's ability of processing auditory information.

In the present study, the subjects performed three tasks in order to identify the infrequent stimulus (MC, PT and RI). Comparing the latency measurements regarding gender and type of task requested to the participant; we observed significant difference for males when MC and PT tasks were compared ($p = 0.0299$) (Table 5).

Regarding the comparison of the measures range with the types of task required to the participant to observe significant difference for PT task (MC – PT $p = 0.0022$ and RI – PT $p = 0.0025$), observed on the Table 3. When comparing gender and task type, only females showed a significant difference when comparing MC and PT tasks ($p = 0.0100$) and RI and PT ($p = 0.0014$), on the Table 5. The comparison between the tasks counting infrequent stimuli and raising one hand when hearing them has already been studied for the measurements of latency and amplitude, with no difference observed between them⁽²⁵⁾. A similar finding was reported when comparing the same measurements to two types of passive tasks, i.e., the task sequencing and an the original task⁽²⁶⁾.

However, reports that task type can influence results of long-latency auditory evoked potentials are present in the literature⁽²⁷⁾. The authors concluded that the complex N1, P2, N2 has not changed, but P300 has showed slight change showing up earlier in the procedure with lower cognitive complexity.

The motor act of raising a hand to identify the perception of the infrequent stimulus has been reported as easiest and it is believed that this task can be adapted for individuals with difficulties in the exam just mentally counting⁽²⁵⁾.

Another factor to consider is the necessity to choose one task type to control the maintenance of the evaluated answer more efficiently. During the recordings, in certain populations, the absence of P3 wave may be due to no control of individual attention (e.g. when you are mentally counting) which can be

easily checked by the researcher to ask the task of “raising the index finger” when the individual hears the infrequent stimulus, linking the motor act to the appearance of infrequent stimulus.

The task with a verbal response is more complex, indicating a need for learning or neuromaturation of the nervous system⁽²⁸⁾. Still according to the author⁽²²⁾, naming, a linguistic activity, demands dependent processes of complex connections of thinking with the language⁽²⁸⁾. The mean values of P300 latencies are higher for verbal than non-verbal stimuli and, conversely, the mean amplitudes are lower for verbal than non-verbal stimuli⁽²²⁾.

For the three studied tasks, the mean lowest latency result was found for task MC (282.88 ms). Significance was observed only when task PT was compared to RI ($p = 0.032$), showing the highest values of latency for the latter (Table 3). These results raise important questions regarding the choice of task to be requested for the participant, since it is a significant factor both for controlling the correct performance of the test, i.e., ensuring that the participant is attentive and that the response (recorded or not), actually depicts the condition of the subject, and the interpretation of the examiner according to the chosen task.

The temporal sequencing task, such as MC, involves both brain hemispheres, with different functions but working together regardless of the stimulated ear⁽²⁸⁾. The right hemisphere may be responsible for the recognition of the acoustic contour and the left one for temporal sequencing and for naming what was heard⁽²⁹⁾. Thus, the difficulty in the naming modality may be explained by the need for interhemispheric integration of the stimuli via the corpus callosum in the request of a verbal response, a fact that did not occur with a non-verbal request⁽³⁰⁾.

CONCLUSION

The mean latency and amplitude values detected were 296.14 ± 15.95 ms and 5.72 ± 0.75 μ V, respectively. The best values of latency and amplitude were obtained when at Cz position, with highest amplitude and latency nearest to 300 ms. However, when associated with gender, there was no variations in these measures between the electrode positions (Fz and Cz). Lowest latencies were obtained for the PT task and the highest amplitudes for the RI task.

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Author contributions

HOS contribution with conception and design of the study, preparation of schedule, literature review, data collection, analysis and interpretation of data, article writing; ACFE contribution with analysis and interpretation of data, article writing and approval of the final version; SZ contributions with analysis and interpretation of data, article writing and critical review of the final version; MAH contribution with: analysis and interpretation of data, article writing and critical review of the final version; ACMBR contributions like advisor professor; principal investigator, conception and design of the study, preparation of schedule, literature survey, collection, analysis and interpretation of data, article writing and approval of the final version.