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Event-related evoked potential P300 in children: reference values

Potencial evocado cognitivo P300 em crianças: valores de referência

Aline Pinto Kropidlofscky[1], *Camila Goldstein Fridman*[2], *Viviann Magalhães Silva Borges*[3], *Dayane Domeneghini Didoné*[4], *Paulo Ricardo Gazzola Zen*[5], *Pricila Sleifer*[6]

ABSTRACT

Purpose: To measure and compare P300 latency and amplitude in normal hearing children at different age groups. **Methods**: A cross-sectional study with 96 children aged between 8 and 11 years and 11 months, equally divided into four age-based groups. P300 assessment was conducted using the Masbe ATC Plus equipment (Contronic®), with 1000 Hz and 2000 Hz stimuli, at an 80/20% probability. **Results**: The mean P300 latency and amplitude values were 339.57 ± 41.03 and 18.22 ± 7.59 in G1, 327.94 ± 37.29 and 16.32 ± 7.01 in G2, 321.05 ± 31.84 and 15.86 ± 5.08 in G3, and 310.76 ± 28.99 and 11.24 ± 3.12 in G4, respectively. **Conclusion**: All children presented P300. There were no differences regarding P300 latencies and amplitudes when comparing different age groups in this study.

Keywords: P300 evoked potentials; Auditory cortex; Electrophysiology; Hearing; Child.

RESUMO

Objetivo: mensurar e comparar a latência e a amplitude do potencial evocado cognitivo P300 em crianças normo-ouvintes, em diferentes faixas etárias. **Métodos**: estudo transversal realizado com 96 crianças com desenvolvimento típico, de 8 anos a 11 anos e 11 meses, divididas em quatro grupos estratificados por idade (G1, G2, G3 e G4). O P300 foi registrado com o equipamento Masbe ATC Plus (Contronic®), utilizando estímulos de 1000 e 2000 Hz (80% frequentes; 20% raros). **Resultados**: o valor médio de latência e de amplitude do P300 no G1 foi de 339,57 ± 41,03 e 18,22 ± 7,59; no G2, de 327,94 ± 37,29 e 16,32 ± 7,01; no G3, de 321,05 ± 31,84 e 15,86 ± 5,08 e no G4, de 310,76 ± 28,99 e 11,24 ± 3,12, respectivamente. **Conclusão**: todas as crianças apresentaram o potencial evocado cognitivo P300. Não houve diferença em relação à latência e amplitude do P300 na comparação das diferentes faixas etárias.

Palavras-chave: Potenciais evocados P300; Córtex auditivo; Eletrofisiologia; Audição; Criança

Study carried out at Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS), Brasil.
[1]Programa de Pós-graduação em Patologia (Mestrado), Universidade Federal de Ciências da Saúde de Porto Alegre – UFCSPA – Porto Alegre (RS), Brasil.
[2]Programa de Pós-graduação Health and Rehabilitation Sciences (Mestrado), University of Western Ontario – UWO – Londres (ON), Canadá.
[3]Programa de Pós-graduação em Saúde da Criança e do Adolescente (Mestrado), Faculdade de Medicina, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS), Brasil.
[4]Departamento de Fonoaudiologia, Universidade Federal de Santa Maria – UFSM – Santa Maria (RS), Brasil.
[5]Departamento de Clínica Médica, Universidade Federal de Ciências da Saúde de Porto Alegre – UFCSPA – Porto Alegre (RS), Brasil.
[6]Departamento de Saúde e Comunicação Humana, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS), Brasil.
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Corresponding author: Viviann Magalhães Silva Borges. E-mail: viviann.msb@gmail.com
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INTRODUCTION

The combination of subjective and objective methods contributes to a more accurate audiological diagnosis and is highly recommended in clinical practice, as it allows the investigation of both function and neurophysiological integrity of the auditory pathways[1,2]. Long latency auditory evoked potentials (LLAEP) deserve to be highlighted within the existing electrophysiological procedures, due to their ability to reflect brain activity corresponding to specific cognitive processes. They are among the most promising tests to assess changes and dysfunctions of the central auditory nervous system[3,4].

Under these circumstances, the evoked cognitive potential P300 stands out. It consists of a positive component in the electrophysiological tracing, generated from the discrimination of rare auditory stimuli among frequent auditory stimuli. It is elicited by different types of events, such as verbal (words or syllables) and non-verbal (pure tone or noise), allowing analyses in both linguistic and non-linguistic contexts. This evoked potential is generated around 300 ms and reflects the activity of brain areas related to cognition, memory, and auditory attention[5-9]. Therefore, it has been used in different populations with developmental disorders that affect intellectual capacity, communication, behavior, hearing, and oral or written language[9-13]. Among these clinical applications, its relevance stands out in the assessment of patients with Central Auditory Processing Disorder (CAPD), enabling the investigation of sensory responses associated with auditory discrimination skills and sustained attention[14]. Thus, the P300 represents a valuable tool for assessing the pediatric population, particularly children with speech-language and auditory needs[9,15-17].

Despite the vast possibility of clinical applications in children, there are still knowledge gaps regarding normative data for P300 parameters. Studies analyzing this potential in typically developing children remain limited. In fact, only a small number of publications with this specific focus have been identified, especially when compared with the much larger number of studies involving adults and older adults[5-8,18].

Previous studies have shown that factors such as age and neural maturation significantly impact P300 findings, highlighting the need for age-adjusted normative standards[5,19]. A large portion of studies aim to investigate broad age groups, revealing a scarcity of research involving younger and more specific ages that examine maturational changes. Considering the clinical relevance and the need for systematization of P300 parameters in the pediatric population, this study is justified because it employs a specific protocol using equipment that has not yet been standardized for children.

Therefore, aiming to provide further insight into the analysis of P300 responses in the pediatric population, the goal of this study is to measure and compare the P300 latency and amplitude in normal hearing children from 8 to 11 years and 11 months of age, divided into different age groups. Thus, suggesting reference values with tone burst stimuli in this population.

METHODS

This is a cross-sectional study. The ethical and methodological aspects of this research were approved by the Research Ethics Committee for Human Subjects (Resolution 466/12) of the Psychology Institute of the Federal University of Rio Grande do Sul (UFRGS), under protocol number 20690. The children were invited to participate in this research through the Informed Assent Form, and they were previously instructed about each procedure that would be performed. Parents or caregivers were informed about the goal, risks, benefits, and confidentiality, and those who agreed to participate signed the Informed Consent Form.

The convenience sample consisted of 96 children, 49 females and 47 males, aged between 8 and 11 years and 11 months, divided into four groups stratified by age: 8.0-8.11 years (G1), 9.0-9.11 years (G2), 10.0-10.11 years (G3), and 11.0-11.11 years (G4). A standardized effect of 0.8, a significance level of 0.05, and a test power of 90% were considered (EpiInfo - Statcal) to estimate the sample size. Thus, a minimum total sample size of 78 individuals was calculated.

Children from public schools, referred to the Study Center for Electrophysiology Testing, Audiology Clinic (UFRGS), were included if they met the following inclusion criteria: aged between 8 years and 11 years and 11 months; regular school performance, confirmed by not having repeated a grade for two consecutive semesters before participation in the study; auditory thresholds within normal limits in both ears[20]; type A tympanometric curve[21]; presence of ipsilateral and contralateral acoustic reflexes at all tested frequencies in both ears; no auditory complaints; no history of otological pathologies (recurrent otitis, tinnitus, or diagnosed hearing disorders), all verified during anamnesis; not using continuous medication; and capable of understanding the procedures required for the examination. Children with genetic alterations or craniofacial abnormalities, or with evident or reported intellectual or neurological deficiency that prevented the performance of an examination, were excluded from the study, in addition to those who did not complete the stipulated evaluations.

First, an anamnesis was conducted to collect general information, including name, age, sex, background/complaint, data on pregnancy, childbirth, psychomotor development, past illnesses, education, learning difficulties, language, and hearing. Before the investigation of auditory evoked potentials, the following procedures were performed: visual inspection of the external auditory canal (EAC) with a Welch Allyn otoscope, pure tone audiometry (PTA), vocal audiometry, and acoustic immittance measures (AIM).

The PTA was performed in an acoustically treated booth, with an Inventis calibrated audiometer (model Harp Inventis) and supra-aural headphones. The air conduction audiometry was carried out in the frequencies of 250, 500, 1000, 2000, 3000, 4000, 6000, and 8000 Hz, and the bone conduction audiometry in the frequencies of 500, 1000, 2000, 3000, and 4000 Hz. To be considered typically hearing individuals, the children should have a pure-tone average of frequencies 500, 1000, and 2000 Hz ≤15 dB HL[20]. Afterwards, vocal audiometry was performed through the Speech Recognition Percentage Index (SRPI) and Speech Recognition Threshold (SRT). To conduct the SRPI, 25 monosyllables were presented in a fixed and comfortable intensity (40 dB HL above the three-tone average for air conduction) in each ear. For SRT, the initial intensity used was also 40 dB HL above the same three-tone average, which was reduced until reaching the level of intensity at which the subject was still able to understand and repeat 50% of the presented trisyllables.

After completion of the PTA, acoustic immitance measurements were performed with Interacoustics equipment (model Impedance Audiometer AT235). A tympanometric curve was obtained by inserting a probe at the entrance of the participant's external auditory canal. Thresholds at frequencies of 500, 1000, 2000, and 4000 Hz were tested in both ears in the search for ipsilateral and contralateral acoustic reflexes. The sample included individuals with type A tympanograms and acoustic reflexes present bilaterally.

After peripheral audiological evaluations, an Auditory Brainstem Response (ABR) test was performed to verify the integrity of the auditory pathway. Click stimulus was presented separately in each ear at a rate of 27.7 stimuli per second, with 2,048 sweeps averaged at an intensity of 80 dB HL. A 12 ms recording window was used, with a 100 Hz high-pass filter and a 3,000 Hz low-pass filter, stimulus duration of 100 µs, and rarefaction polarity. To ensure reproducibility, two recordings were obtained for each ear. The absolute latencies of waves I, III, and V were considered within normal limits if ≤1.54 ms, ≤3.69 ms, and ≤5.54 ms, respectively, and interpeak intervals were considered within normal limits if I–III ≤2.14 ms, III–V ≤1.86 ms, and I–V ≤4.00 ms[22].

Subsequently, the P300 recording was performed. The test was conducted in a quiet, acoustically and electrically treated room, in which the individual was comfortably positioned on a chair with a headrest. The examiner prepared the skin by cleaning it with an abrasive agent, alcohol, and sterile gauze. Next, silver electrodes were attached using electrolyte paste (Ten20) and micropore tape: on the forehead (Fpz for the ground electrode), at Fz (active electrode), and on the left (M1) and right (M2) mastoids, according to the international 10–20 system. ER-3A insert earphones were used, and P300 recordings were performed using the Masbe ATC Plus system (Contronic®). Electrode impedance was considered acceptable if below 5 Ω for each derivation, with inter-electrode differences not exceeding 2 Ω. The electroencephalogram (EEG) was recorded to capture spontaneous brain electrical activity and to identify artifacts that could interfere with the examination.

In order to register the P300, a sequence of equal stimuli (frequent stimulus) was presented with short intervals of time, alternated with stimuli that differed in frequency (rare stimulus). Before starting the exam, the participants were instructed and conditioned to the mental counting task, which consists of reporting the number of rare stimuli detected in the total sequence of stimuli. Training was performed to verify the understanding and correct discrimination of the acoustic stimuli. The stimuli were presented using insertion earphones, and the intensity of the stimuli was 80 dB HL. Finally, the subjects were asked about the quantity of rare stimuli they had heard, and the response was compared to the number of rare stimuli recorded by the equipment.

The P300 was conducted monaurally, with tone burst stimuli at the frequency of 1000 Hz (50 cycles) for the frequent stimulus, and 2000 Hz (100 cycles) for the rare, with a 20% rise and decay time with a trapezoidal envelope. These were presented in an oddball paradigm, with a probability of 20% and 80% of appearance. The stimuli were presented at a rate of 0.8 pulses per second (pps). At the time of acquisition, the full scale was 200 µV, a 1 Hz high-pass filter, a 20 Hz low-pass filter, and a 1000 ms time window. The research protocol employed in this study was adapted from a protocol previously proposed for this equipment[23].

Three recordings were obtained from each ear to verify the reproducibility of the waves. For the wave selection, the highest peak of positive polarity after the N1-P2-N2 complex was considered. It should be noted that, in order to ensure greater reliability in the analyses, all electrophysiological records were analyzed by two judges with experience in electrophysiology of hearing at different times. The results were considered valid only when there was an agreement between the analyses. To verify this agreement, Kappa's statistical methods were used[24]. The correlation between the strength of agreement and the Kappa value was interpreted based on the scale: <0.00 (poor), 0.00-0.20 (negligible), 0.21-0.40 (weak), 0.41-0.60 (moderate), 0.61-0.80 (substantial), and 0.81-1.00 (almost perfect). The interpretation of the Intraclass Correlation Coefficient (ICC) was based on the following classification: ICC < 0.4 (poor correlation), 0.4 ≤ ICC ≤ 0.75 (satisfactory correlation), and ICC > 0.75 (excellent correlation)[25].

After collecting the data, the results were displayed in Microsoft Excel spreadsheets and analyzed using the Statistical Package for Social Science (SPSS) software, version 21.0. They were organized in the form of descriptive statistics. The Kolmogorov-Smirnov test was used to assess the normality of the data. Quantitative variables were described using mean and standard deviation, while qualitative variables were presented as absolute and relative frequencies. Student's t-tests were performed to compare latency and amplitude results between ears and sexes. Group comparisons were performed using the nonparametric Mann–Whitney test, with a significance level of 5% ($p < 0.05$).

RESULTS

From the 104 children originally invited to participate in this research, eight were excluded for not completing the proposed procedures or for not meeting the inclusion criteria. Thus, the results refer to a sample of 96 children, divided into four groups stratified by age. Table 1 shows sample characterization.

Excellent inter-rater agreement was observed for P300 latency and amplitude analyses (Kappa = 0.85), with an intraclass correlation coefficient of 0.82, indicating near-perfect reliability according to standard criteria.

The mean latency and amplitude values of the P300 were: in G1, 339.57 ± 41.03 ms and 18.22 ± 7.59 µV; in G2, 327.94 ± 37.29 ms and 16.32 ± 7.01 µV; in G3, 321.05 ± 31.84 ms and 15.86 ± 5.08 µV; and in G4, 310.76 ± 28.99 ms and 11.24 ± 3.12 µV, respectively.

In the comparative analysis between right (RE) and left (LE) ears, no statistically significant difference was found for latency ($p = 0.251$) or amplitude ($p = 0.428$) between groups, indicating that the RE and the LE have equivalent P300 latency and amplitude values, so the results were combined. There were also no statistically significant differences in P300 latency or amplitude values between sexes ($p = 0.373$ and $p = 0.296$, respectively). Consequently, no evidence was found that P300 latency and amplitude values in children are affected by sex, and the data were also analyzed collectively.

Although differences were observed in the mean and standard deviation of P300 latency and amplitude for each group, these were not statistically significant. However, a negative correlation was found between the variables age, latency, and amplitude. That is, the older the child, the lower the P300 latency and amplitude in both ears (Table 2).

Table 1. Sample characterization considering age and sex

Variables	n= 96
Age (years) – mean ± SD [min – max]	9.6 ± 0.9 [8 – 11]
Group	Age (n)
G1	8 years to 8 years and 11 months (24)
G2	9 years to 9 years and 11 months (24)
G3	10 years to 10 years and 11 months (24)
G4	11 years to 11 years and 11 months (24)
Sex	n (%)
Male	47 (48.95)
Female	49 (51.04)

Subtitle: n = sample number; SD = standard deviation; min = minimum; max = maximum; % = corresponding percentage of sample number

Table 2. Results of P300 latencies and amplitude analyses in each group

Variables	Group 1 (n=24) Mean ± SD	Group 2 (n=24) Mean ± SD	Group 3 (n=24) Mean ± SD	Group 4 (n=24) Mean ± SD	p* value
P300 Latency (ms)	339.57±41.03	327.94 ± 37.29	321.05 ± 31.84	310.76 ± 28.99	0.097
P300 Amplitude (μV)	18.22 ± 7,59	16.32 ± 7.01	15.86 ± 5.08	11.24 ± 3.12	0.125

Student t-test; p≤0.05 significant

Subtitle: n = sample number; SD = standard deviation; ms = millisecond; μV = microvolt

DISCUSSION

Among the parameters analyzed, latency is considered to show the greatest variability when data are examined by age. Latency reflects the time course of processing activity in milliseconds[3], and values for the pediatric population are higher than those found in adults[23], since the maturation of the auditory pathway influences latency parameters as a function of age[5-7]. P300 latency ranges from 241 to 396 ms in children aged 5 to 12 years, indicating that children as young as 5 to 7 years already present P300 responses, although with prolonged latencies, reaching maturity around adolescence[3].

The average latency value obtained between the four groups (8.0-11.11 years) in this study was 324.83 ms. This finding is consistent with the results of other studies reporting mean P300 latencies between 326.8 and 382.7 ms[5,15,17] in children of the same age range. It was generally observed that, although variation exists between studies, a decrease in latency values is commonly noted with increasing age. The wide variability of P300 responses is an important consideration and could be explained by the use of different protocols and equipment. Moreover, despite being an objective test, several factors can influence its findings, such as the patient's age, attention, time of testing, stimulus counting method, among others[3,23].

The LLAEPs are known to have specific indications and criteria for the pediatric population due to the auditory pathways maturation process, considering that the amplitude of responses depends on the magnitude of synapses that occur in the cerebral cortex[4,7,26]. Changes in P300 amplitude associated with puberty have also been described in the literature, suggesting the influence of neurobiological development on the morphology of this potential[27]. Therefore, age is an important factor to be considered when interpreting the values obtained in the P300 test. Such arguments justify the stratification by age made in the present study, allowing the assessment of cognitive development across different age groups and the establishment of age-specific reference values.

Although relevant, the analysis of age effects in typically developing children remains scarce in the literature, with only a few studies identified that considered stratification by age year[5,17]. The majority of studies focus on adults and older adults, children with neurodevelopmental disorders, or use very broad age ranges, which hinders the interpretation and generalization of findings[28].

Another limitation observed in the literature concerns the sample sizes, as most studies with control groups include a small number of children in their samples[8-11,14,16]. Given the considerable variability of P300 responses, studies with a larger number of participants are recommended. Therefore, the current study's results may contribute to the accuracy of normative values for the investigated age range, as it includes a larger sample.

In addition to latency, another parameter analyzed is the P300 amplitude. Related to the event or task that elicits the response, amplitude reflects the allocation of neural resources during cognitive processes[3,7]. Studies suggest that there is an approximate decrease of 0.2 μV per year[29] in P300 amplitude. However, considerable variability has been observed, with values ranging from 1.7 μV to 19 μV[3,7]. In the present study, the mean value obtained across groups was 15.41 μV, which is close to the upper limit described in the literature and comparable to findings reported in studies with children of the same age range[9,11,15].

Although no statistically significant difference was found, there was a reduction in the amplitude values with the increase in age. According to the literature, changes in P300 amplitude from childhood to adulthood may reflect differences in the stage of functional brain development. A literature review[19] highlights findings from studies showing that increased skull thickness, as well as greater distance between the brain and the scalp, can result in lower P300 amplitudes, precisely because they hinder the assessment of regions associated with the potential response.

The relationship between electrode placement and amplitude has been mentioned by other authors[29], who recommend recording from electrodes positioned over the central and parietal gyri (Cz and Pz) to achieve better latency and amplitude results.

However, there are few studies linking the auditory P300 to imaging exams that allow a deeper understanding of maturational development, highlighting the need for further scientific investigation into the relationship between the P300 and brain structure during development.

CONCLUSION

The present study successfully identified the cognitive P300 potential in all evaluated children, providing normative values for both latency and amplitude in children aged 8 to 11 years and 11 months. These findings, consistent with previous reports, may serve as an important clinical reference for the interpretation of P300 responses in pediatric populations. The limited number of normative studies in this field highlights the relevance of the current study, emphasizing the need for standardized protocols and further research. In particular, longitudinal studies are recommended to deepen our understanding of the maturational development of the central auditory system and its impact on cognitive auditory processing throughout childhood.

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