Arq Neuropsiquiatr 2004;62(3-A):575-581

VISUAL EVENT-RELATED POTENTIAL (P300)

A normative study

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ABSTRACT - The P300 component of the Event-Related Potential (ERP) is a general measurement of "cognitive efficiency". It is an index of the ability of an individual's Central Nervous System (CNS) to process incoming information. *Objective:* To develop a normative database for the visual P300. *Methodology:* 30 right-handed individuals (same number of each sex), between 20 and 30 years of age, healthy, free of any cognitive impairment and not making use of psychoactive substances. Participants were submitted to a visual discrimination task, which employed the "oddball" paradigm. *Results:* The expected scalp distribution trend was seen for latency but not for amplitude values. *Conclusion:* A high variability of latency and amplitude values was observed across the age span. Mean reaction time for the entire sample of the study was 391.56 $\pm$ 37.03 ms.

KEY WORDS: event-related potential, P300.

Potencial evocado visual relacionado a evento (P300): estudo normativo

RESUMO - O componente P300 do Potencial Evocado Relacionado a Evento é uma medida geral de "eficiência cognitiva" e um índice da qualidade do processamento e armazenamento de informações pelo sistema nervoso central. *Objetivo:* Desenvolvimento de um banco normativo do P300 visual. *Metodologia:* 30 sujeitos destros (ambos os sexos), entre 20 e 30 anos de idade, sadios e livres de qualquer déficit cognitivo. Os sujeitos não estavam fazendo uso de substâncias psicotrópicas ou psicoativas e foram submetidos a uma tarefa de discriminação visual utilizando o paradigma "oddball". *Resultados:* O padrão de distribuição cortical esperado foi observado para os valores de latência, mas não para os de amplitude. *Conclusão:* Foi observada grande variabilidade dos valores de latência e amplitude no grupo analisado. O tempo de reação médio da amostra foi 391,56 $\pm$ 37,03 ms.

PALAVRAS-CHAVE: potencial evocado relacionado a evento, P300.

In the late 1960's, a careful analysis of the electroencephalogram (EEG) revealed that the presentation of a stimulus produces specific changes in the brain[1]. During the presentation of a stimulus, there is a significant increase in synaptic activity in millions of neurons simultaneously. Changes in the membranes' potentials occur in a fraction of a second, after the stimulus is presented, in distinct regions of the brain. Since these synaptic potentials are evoked by a stimulus, they occur in a synchronized way. The combined electrical responses of this neuronal population are known as Evoked Potentials[2]. The Evoked Potentials (EP) or Event-Related Potentials (ERP) consist of a series of positive and negative waves that can be named numerically or according to their latency. For example, the third positive wave of the ERP is named P3 or P300. The main ERP waves are: N1, P2, N2, and P3. Although all the waves can be recorded and analyzed, the focus of most clinical studies has been the P300. Specifically, ERP can be understood as manifestations of specific psychological processes[3]. A

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Received 30 September 2003, received in final form 20 February 2004. Accepted 16 March 2004.

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distinction must be made between the different types of ERP's. Potentials whose characteristics are controlled by the physical properties of the stimulus are classified as "exogenous". On the other hand, potentials whose characteristics are determined by the nature of the interaction between the individual and the stimulus are classified as "endogenous". The Endogenous Evoked Potentials are long-latency potentials related to aspects of cognitive processing[4]. For this reason, they became the focus of studies related to cerebral functioning.

The ERP has demonstrated reasonable success among the various electrophysiological techniques as a means to assess disturbances in cognitive function. Specifically, this neuroelectric measure has played a crucial role in the quantification and understanding of age-related cognitive changes[5]. The mapping of EP provides direct evidences of the relationship between the integrity of the central nervous system (CNS) and aging[6]. During the aging process, CNS function slows down due to neurobiological modifications[7]. Along this vein, the P300 component of the EP becomes a valuable tool in the study of this process, because it is thought to result from neural activity associated with attentional and memory processes[8,9]. Once the P300 is evoked by stimuli that require a higher level of cognitive processing, it provides clear evidence of the significant slowing down of the CNS during normal and pathological aging processes[10,11].

The need to develop a normative database is mostly due to the lack of a specific P300 database for the Brazilian population. The need to develop such database is also due to the great result variability within a subject and among subjects, caused by different variables, such as: type of task employed, subject's psychophysiological state, attention allocation at the exact moment of the visual stimulus display, among others. To achieve this goal, two variables are used in the quantification of the P300: latency, which reflects the time required to allocate resources and engage memory updating for a given task, and amplitude, which indexes attentional resource allocation for immediate memory processes. Individual differences in P300 values provide a reliable indication of the variability in neuroelectric processing capability and speed of the brain's attentional and mnestic mechanisms. The P300 component can assay the mental changes brought on by normal aging and cognitive diseases, as well as motor disturbances[12,13] associated with information processing. Therefore, the development of a normative database provides a more precise evaluation of psychiatric, neurological and motor disorders and their impact on cognitive functions[14,15]. Considering that the definition of normal component values and the most appropriate method for their acquisition have still not been well characterized, the development of a database becomes essential in the process of enhancing the usefullness of this neurophysiological tool.

METHOD

Subjects - The sample consisted of 30 individuals, 15 male and 15 female, with ages ranging from 20 to 30 years. Subjects were selected among undergraduate and graduate students from different institutions in Rio de Janeiro. All subjects were healthy, free of cognitive deficits and were not making use of any psychoactive or psychotropic substance at the time of the test. To assure that subjects did not present any impairment of their physical and mental health, and to identify and exclude from the experiment any subjects who could contaminate future results, a questionnaire was applied. The questionnaire also aimed at identifying possible P300 biological determinants, such as food intake, body temperature, fatigue, drugs, among others. Laterality was used as an exclusion criterion. The Edinburgh Inventory[16] was used to assess laterality and exclude left-handed individuals from the experiment. Subjects signed a consent form, where the experimental condition was thoroughly described. The experiment was submitted to the Psychiatric Institute's ethics committee for approval.

Experimental procedures - A sound-attenuated room was prepared for data acquisition. Subjects were seated comfortably in a chair with arm-rest to minimize muscular artifacts. During the visual task, lights were turned off for subjects to concentrate exclusively on the monitor screen. A 15" Samsung monitor was placed in front of the individual. The visual stimulus was presented on the monitor by the ERP acquisition software, developed in DELPHI 5.0.

To elicit the P300, all subjects were submitted to the same visual discrimination task, which employed the "oddball" paradigm. In this paradigm, two stimuli are presented randomly, with one occurring infrequently[17]. The subjects were asked to discriminate target (infrequent) from non-target or standard stimuli (frequent). In the present experiment, target stimuli were represented by a square and non-target, by a circle. Subjects were instructed to respond to the target stimulus by pressing a button in a joystick (Model Quick Shot-Crystal CS4281). The joystick was used to measure individuals' reaction time at each trial. Although reaction time is independent from ERP measures, it was used to verify subjects' alertness during the task. Each subject was submitted to two blocks of 100 trials each. In other words, the square was presented 100 times in each block. The stimulus

appeared on the screen for 0.75 seconds, with the same time interval between stimuli.

Data acquisition - International 10/20 System[18] for electrode placement (referred to linked earlobes) was used with a 20-channel Braintech-3000 (EMSA-Medical Instruments, Brazil). The 19 monopolar electrodes were arranged in a nylon cap (ElectroCap Inc., Fairfax, VA). Impedance for EEG and EOG electrodes were under 5 KΩ and 20 KΩ, respectively. Visual inspection was employed for detection and elimination of artifacts. The data acquired had total amplitude of less than 100 μV. The signal was amplified with a gain of 22,000. The EEG signals were acquired between 0.01 and 50 Hz. Eye-movement (EOG) artifact was monitored with a bipolar electrode montage using two 9-mm diameter electrodes attached above and on the external canthus of the right eye. Moreover, Independent Component Analysis (ICA) was applied to remove possible sources of artifacts. The EEG signal was analogically filtered between 0.01 Hz (high-pass) and 100 Hz (low-pass), and sampled at 240 Hz. The software *ERP Acquisition* (Delphi 5.0), developed at the Brain Mapping and Sensorimotor Integration Lab, was employed with the following digital filters: Notch (60 Hz), high-pass of 0.3 Hz and low-pass of 25 Hz.

Average processing - The program *Average* (MATLAB 5.3), which implements filter and epoch selection routines, was used to process acquired data. After data were acquired and stored, the average software loaded the data and established different routines. Specific filters were set up: a high-pass filter of 0.1 Hz and a low-pass of 20 Hz. The target stimulus (square) was selected as the trigger-stimulus. Epochs (i.e., visualization windows) were set to begin at the time of stimulus onset until 700 ms after. After specific channels were selected (Fz, Cz, and Pz), data were averaged and represented graphically in terms of latency (x-axis) and amplitude (y-axis).

Component analysis - The P300 component was identified as the most positive component within the latency window of 250-500 ms. Amplitude was measured relative to a pre-stimulus baseline, with peak latency defined as the time point of maximum positive amplitude within the specific latency window.

Statistical analysis - In order to develop the normative database, the following statistical analyses were applied: 1) The average of each subject (i.e., 100 trials/block) was calculated individually for amplitude and latency values, in Fz, Cz, and Pz electrodes separately; 2) The average of blocks I and II was then calculated for each subject for the same variables (i.e., amplitude and latency) and in the same electrode sites; 3) A descriptive statistical analysis (mean/sd) was made for the entire sample of the study (20-30 years), following the same parameters specified above; 4) A linear regression analysis was applied to the amplitude and latency variables separately, in relation to age, to describe the rate of decrease (amplitude), increase (latency), and dispersion along the specific age span, in the three electrode sites; 5) P300 amplitude and latency were then assessed by a two-way ANOVA (age group x electrode site). The distinct age groups were divided as follows: 20-23, 24-27, and 28-31 years. A Post Hoc (Scheffé) was applied a posteriori; 6) A one-way (electrode site) ANOVA, followed by a Post Hoc (Tukey) test, was performed for latency and amplitude values, in Fz, Cz, and Pz electrode sites, to verify whether electrodes were significantly different; 7) Finally, individual Reaction Time averages (blocks I and II) were collapsed, yielding a great mean for the entire sample of the study.

RESULTS

Figure 1 illustrates the grand mean for the entire sample of the study in each electrode site. Fig. 1-A shows an increment in latency values: Fz = 366.63 ± 26.29 ms, Cz = 370.24 ± 25.04 ms, Pz = 387.70 ± 20.16 ms. ANOVA results indicated a significant difference across electrode sites (p = .002). The Post Hoc (Tukey) analysis revealed differences between Fz-Pz (p = .003) and Cz-Pz (p = .016). Fig. 1-B illustrates amplitude variations across electrode sites: Fz = 2.87 ± 1.43 μV, Cz = 2.74 ± 1.27 μV, Pz = 2.98 ± 1.33 μV. No significant differences were observed (p = .790).

Figure 2 represents the linear regression analysis applied to the amplitude and latency variables separately, in relation to age. The plot describes the rate of decrease (amplitude), increase (latency), and dispersion along the specific age span, for each electrode site separately. Latency R values are: Fz (R = .3281), Cz (R = .2988) and Pz (R = .0547). Amplitude R values are: Fz (R = .4068), Cz (R = .3324) and Pz (R = .2437). The figure also shows linear equations for the regression analysis.

Figure 3 expresses latency and amplitude variations in Fz, Cz and Pz, across three distinct age groups: 20-23, 24-27 and 28-31 years. The two-way ANOVA revealed no interaction between age group and electrode site (p = .909) for latency. No main age group effects were found (p = .258). However, the analysis demonstrated a significant effect of electrode site (p = .009). The Post Hoc (Scheffé) analysis confirmed previous results, which pointed out to a difference between Pz and the others (i.e., Fz and Cz). In relation to amplitude values, the two-way ANOVA showed no interaction between age group and electrode site (p = .610) and no main effects for both age group (p = .973) and electrode site (p = .535).

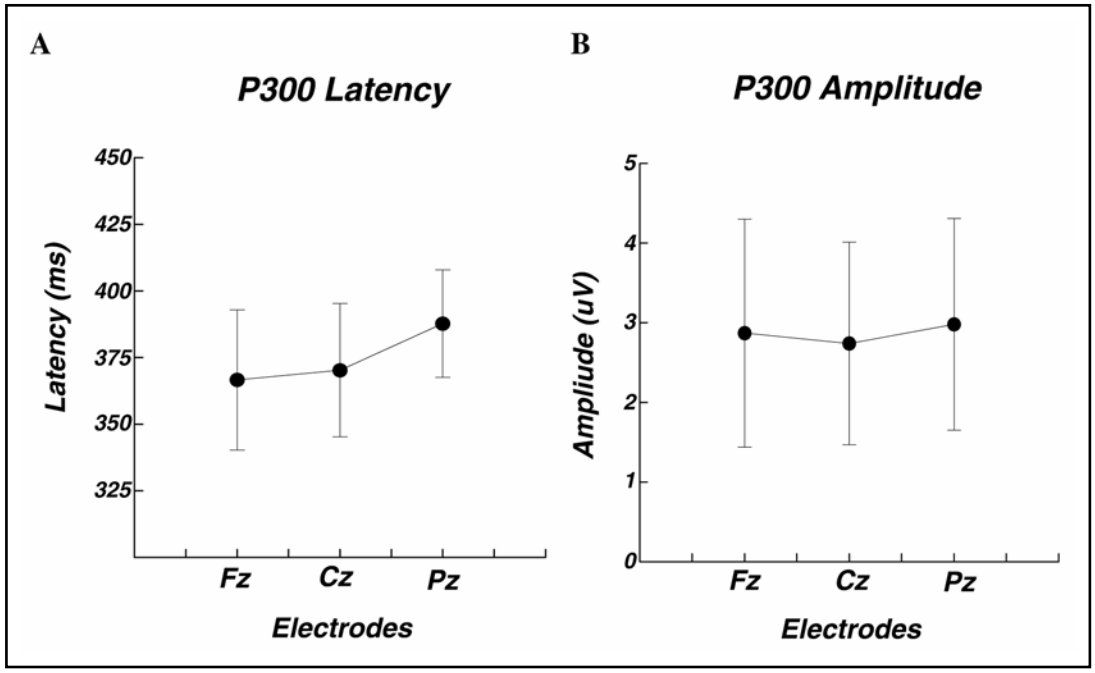

Fig 1. Grand mean for the entire sample of the study (20-30 years), for latency (A) and amplitude (B) values, in Fz, Cz and Pz electrode sites.

As specified previously, mean reaction time was calculated to ensure subjects' alertness during the visual task. Mean reaction time for the entire sample of the study was 391.56 ± 37.03 ms.

DISCUSSION

The present study aimed at developing a P300 normative database. This ERP component has proven to be a valuable asset to cognitive research as a neuroelectric index of age-related changes. P300 analyses indicate the way in which brain processes information. In this sense, a normative database is crucial for the comparison between normal subjects and distinct patient populations, providing a more precise evaluation of the impact of psychiatric, neurological and motor disorders on cognitive functions. It is known that with increased age, there is a change in the speed with which the nervous system responds to external stimuli[19]. In other words, as adults age, there is a slowing of neural transmission time, which results in cognitive disfunctions[8,17]. Specifically, an increase in latency and a decrease in amplitude are observed in elderly adults. Furthermore, it is also known that neuro-degenerative diseases affect the anatomy of the brain, and consequently, its function[20]. Common neuro-degenerative diseases, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis often increase P300 latency. Moreover, studies have reported that some pharmaceuticals affect the latency of ERP's[21]. These substances may directly affect the transmission speed of neurons or the subject's alertness and, thus, affect component values[22,23]. In this sense, P300 becomes a useful neuroelectric measure for evaluating therapeutic strategies involving CNS medications.

Although normative studies have shown an increase in latency and a decrease in amplitude in a given age span[11,24,25], the present results did not confirm this pattern for the specific sample used in the study (i.e., 20-30 years). The dispersion around the regression line (Fig 2) indicates a considerable variability of both latency and amplitude values. Specifically, the linear regression has high variability and a low level of predictability for the single age decade analyzed. This low level of predictability was constant across electrode sites, suggesting that only one decade is not sufficient to produce changes in P300 latency and amplitude, even though P300 latency showed less variability than amplitude. Moreover, when the age span was divided into three groups (i.e., every three years) (Fig 3), no interaction between age group and electrode site was found in either variable (i.e., latency and amplitude). This finding can be explained by the fact that the pattern of amplitude and latency distribution across different electrodes occurs independently from the individuals' age. This fact also explains

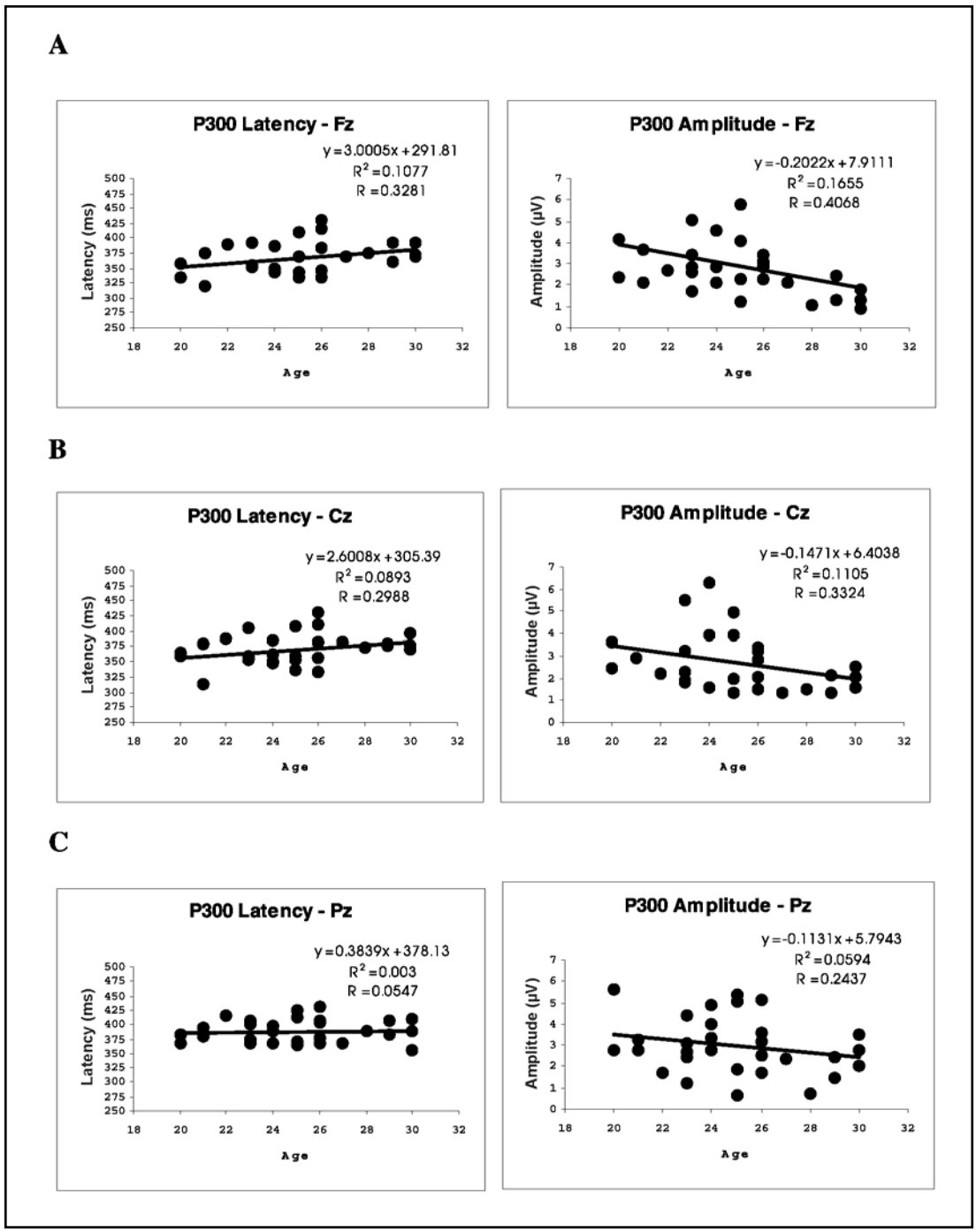

Fig 2. P300 latency and amplitude as a function of age, in Fz (A), Cz (B), and Pz (C) electrode sites. The scattergram describes the rate of decrease (amplitude), increase (latency), and dispersion along the specific age span (20-30 years).

why the only main effect was found for electrode site. The absence of a main age group effect is related to the study's design, which analyzed one single decade, as discussed above.

Previous studies have reported that P300 latency increases from the anterior to the posterior scalp areas, i.e., from Fz (frontal), to Cz (central), and Pz (parietal) electrode sites [8,17,26]. This trend was observed in our results (Fig 1-A). Significant differences were observed between Fz and Cz in relation to Pz. However, the amplitude distribution trend described in the literature was not found. For normal young adults, P300 amplitude increases from the

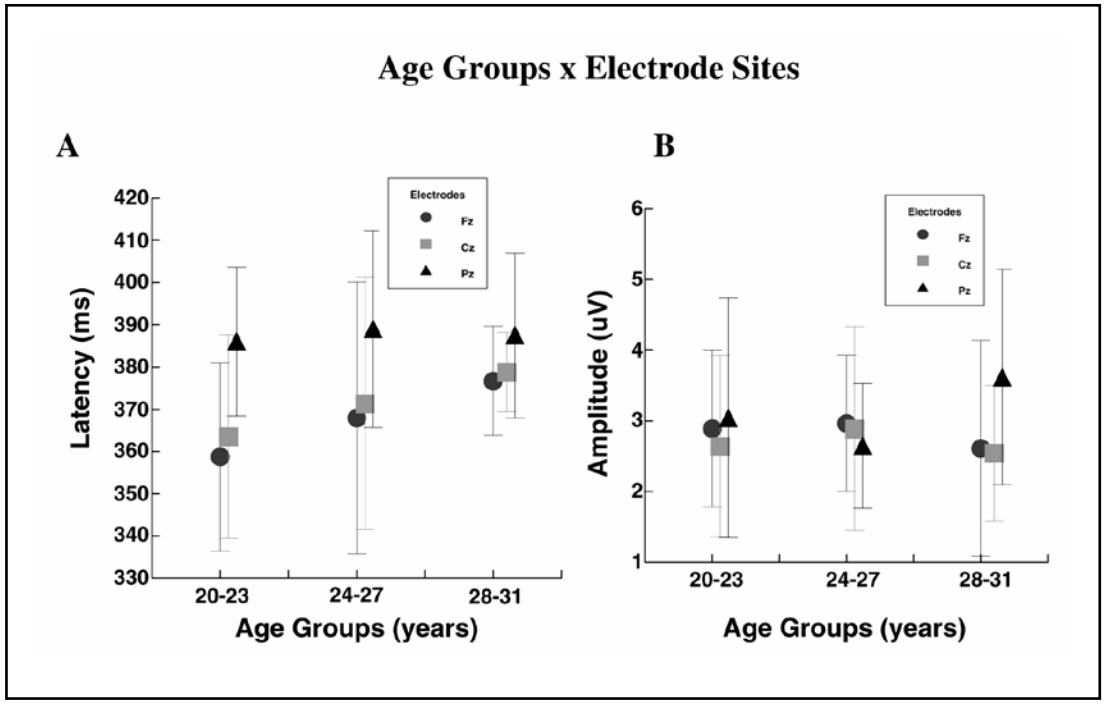

Fig 3. Mean P300 latency and amplitude variations across distinct age groups, in Fz, Cz and Pz electrode sites.

anterior to the posterior scalp areas[8]. The trend observed in the present study is shown in Figure 1-B. The result of the statistical analysis indicated that, despite the contradictory trend, the differences between the amplitude values in the three electrode sites were not significant. Furthermore, amplitude values were similar to previous experiments[27], where the oddball paradigm was employed with a visual task. Ambiguous amplitude patterns have been reported by different laboratories, which can be explained by the specific variables used in the studies. With respect to reaction time, our result is also in agreement with the literature, which states that P300 latency is generally unrelated to response selection processes and independent of behavioral reaction time[28]. Once latency reflects the processing time prior to the motor response, the mean latency at Pz (i.e., 387.70 $\pm$ 20.16 ms), where the P300 component is more prominent, should be shorter than the mean reaction time (i.e., 391.56 $\pm$ 37.03 ms). Our findings support this hypothesis.

A large variability is observed within a subject and among subjects. According to Polich[8], P300 biological determinants such as body temperature, food intake, drugs, and handedness, affect latency and amplitude values. Interstudy variability is also observed. Such variations may be explained by the employment of different parameters, which will consequently yield different results. For example, visual and auditory stimuli produce distinct amplitude and latency values[29]. Moreover, the oddball paradigm produces increased latency and amplitude values when compared to the single stimulus paradigm[8,17]. Along this vein, the specific parameters employed in a study, such as: task (i.e., nature of the response, difficulty), paradigm, stimulus factors (i.e., modality, duration, intensity), software, sample characteristics (i.e., size, density of subject numbers within each age decade, proportions of male and female subjects), among others, will directly influence its results[26]. Therefore, every ERP study will have a singularity and a specificity that may explain possible controversial results[30]. In this context, the P300 normative database developed in the present study is particular for the conditions employed and for the sample selected. Further studies, with larger samples, are necessary to expand the age span and thus, make the P300 normative database even more reliable in the assessment of disorders that impact cognitive capability.

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