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# NEUROMODULATORY EFFECTS OF CAFFEINE AND BROMAZEPAM ON VISUAL EVENT-RELATED POTENTIAL (P300)

## A comparative 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 compare the neuromodulatory effects of caffeine and bromazepam on the visual ERP (P300), in relation to a P300 normative database. *Method*: 15 right-handed individuals (7 male and 8 female), between 20 and 30 years of age, healthy, free of any cognitive impairment and not making use of psychoactive substances were studied. Participants were submitted to a visual discrimination task, which employed the "oddball" paradigm, after the administration of caffeine and bromazepam, in a randomized, double-blind design. *Results*: Statistically significant differences were observed when the caffeine and bromazepam conditions were compared to the normative database. *Conclusion*: The present results suggest that caffeine and bromazepam have distinct modulatory effects on CNS functioning.

KEY WORDS: event-related potential, P300, caffeine, bromazepam.

**Efeitos neuromoduladores da cafeína e do bromazepam no potencial evocado visual relacionado a evento (P300): estudo comparativo**

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 (SNC). *Objetivo*: Comparar os efeitos neuromoduladores da cafeína e do bromazepam a partir do banco normativo do potencial evocado visual (P300). *Método*: 15 sujeitos destros (7 homens e 8 mulheres), entre 20 e 30 anos de idade, sadios, livres de qualquer déficit cognitivo e sem uso de substâncias psicotrópicas ou psicoativas foram estudados. Os sujeitos foram submetidos a uma tarefa de discriminação visual utilizando o paradigma "oddball", após a administração de uma cápsula de cafeína ou de bromazepam, em um desenho duplo-cego randomizado. *Resultados*: Foram observadas diferenças estatisticamente significativas quando as condições cafeína e bromazepam foram comparadas com o banco normativo. *Conclusão*: Os resultados sugerem que a cafeína e o bromazepam têm efeitos moduladores específicos no SNC.

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

Neuroelectric techniques have provided direct evidences of central nervous system (CNS) functioning[1]. Specifically, event-related potentials (ERPs) have become an important index of CNS ability to process incoming information. The P300 component of the ERP is considered a general measurement of "cognitive efficiency"[2]. In this context, it reflects CNS activity related to cognitive operations and helps to discriminate the effects of CNS stimulant and depressor drugs on brain dynamics[3].

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*Caffeine* – Caffeine is an acknowledged CNS stimulant and a widely used psychoactive substance. Although the effects of caffeine on cognitive performance have been subject of numerous experiments, the findings are still not conclusive[4-6]. In this sense, the mechanisms of caffeine's ergogenic effects are not well understood. Partial results suggest that low doses of caffeine (60 mg) affect key aspects of cognitive function related to alertness, vigor, mood, efficiency, and perception of energy[7]. Caffeine has also been associated with improvement in motor performance[8-10]. In this context, caffeine can be regarded as a neuromotor modulator. Moreover, a decrease on reaction time and changes on P300 latency[11,12] and amplitude[13] have been associated with caffeine's modulatory effects. In a recent study, a shorter P300 latency and higher amplitude, specifically at Fz, were observed after the administration of caffeine when compared to placebo. These findings suggest that the tendency of caffeine to improve cognitive performance is probably associated with changes in the frontal cortex, a widely recognized attention area[14]. Given some inconsistencies and contradictions in the current literature, the effects of caffeine on ERPs have not been entirely clarified.

*Bromazepam* – Benzodiazepines, such as bromazepam, have been used in the pharmacological treatment of anxiety since the early 60's[15]. The benzodiazepine family of depressants is used therapeutically to produce sedation, induce sleep, relieve anxiety and muscle spasms, and to prevent seizures. In general, benzodiazepines act as hypnotics in high doses, anxiolytics in moderate doses, and sedatives in low doses. Their mechanism of action on the CNS is believed to be related to their ability to enhance the activity of gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter[16-18] .

Effects of oral doses of bromazepam on memory, psychomotor activity, reaction time and vigilance performance have been widely demonstrated[19-22]. Despite the vast number of vigilance studies that employed benzodiazepines, few have employed bromazepam and ERP measures. One of the few studies that examined the effects of bromazepam (6 and 12 mg) on the P300 component of the ERP in a visual vigilance task was conducted by Leeuwen et al. They observed smaller P300 amplitudes after the administration of the drug. Apparently, bromazepam deteriorates the ability of the individual to detect relevant information in the environment[23].

Studies combining caffeine, bromazepam and P300 are practically inexistant in current literature. Thus, the present study aimed at investigating the distinct neuromodulatory effects of theses drugs on the visual ERP (P300), by comparing the results with a P300 normative database[24].

## METHOD

*Subjects* – The sample of the present study consisted of 15 individuals, 7 male and 8 female, with ages ranging from 20 to 30 years. The P300 normative database sample consisted of 30 individuals, 15 male and 15 female, with the same age range. Subjects of both samples were selected among undergraduate and graduate students from different institutions in the city of 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[25] 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.

*Study design and procedures* – Subjects received a capsule (400 mg of caffeine or 3 mg of bromazepam) on two different occasions under a randomized, double-blind design. The procedures consisted of a two-day treatment: caffeine (C) and bromazepam (B). The procedures were standardized in the following routine: 1) Completion of questionnaire and Edinburgh inventory; 2) Administration of capsule (caffeine or bromazepam); 3) Visual ERP, 30 minutes after drug ingestion. It must be stressed that there was no baseline ERP. In other words, subjects were not submitted to the task before drug ingestion because the P300 normative database would be used as the baseline condition. The P300 normative database was chosen as the baseline condition due to its significant sample size and because sample characteristics were similar to the ones of the present study.

*Visual event-related potential (P300)* – 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 pre-

sented 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[26]. 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[27] 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, USA). 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* – One-way Anova was performed for the reaction time variable, across the two experimental conditions, i.e., caffeine (C), and bromazepam (B), and the P300 normative database (ND). Two-way Anova, condition x electrode (3 x 3), was performed for the electrophysiological measure, i.e., P300 latency and amplitude, in the Fz, Cz, and Pz electrode sites separately. A Post hoc (Scheffé) was applied a posteriori.

## RESULTS

*Behavioral* – Figure 1 illustrates reaction time variations across experimental conditions: ND (378.47 ± 31.10 ms), C (382.46 ± 52.95 ms) and B (397.84 ± 40.07 ms). The statistical analysis did not indicate any difference among the conditions ($p = 0.424$).

*Electrophysiological* – Figure 2 illustrates P300 latency (A) and amplitude (B) variations across conditions (ND, C, B) and electrodes (Fz, Cz, Pz). The two-way Anova revealed no interaction between condition and electrode site ($p = 0.970$) for latency. However, the analysis demonstrated a significant main effect for condition ($p = 0.000$) and electrode site ($p = 0.009$). For condition, the post hoc (Scheffé) analysis pointed out to a difference between ND and C ($p = 0.000$), ND and B ($p = 0.005$), and C and B ($p = 0.002$). For electrode site, the post hoc indicated a difference between Fz and Pz ($p = 0.014$). Mean latency values for each condition were: ND

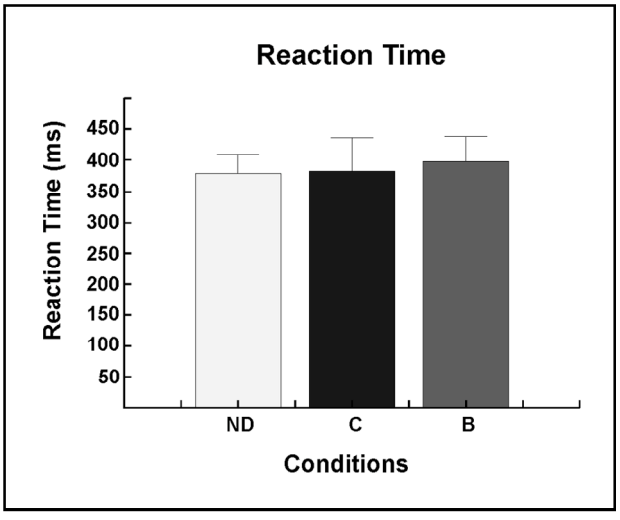


*Fig 1. Reaction time variation across the normative database (ND), caffeine (C), and bromazepam (B) conditions.*

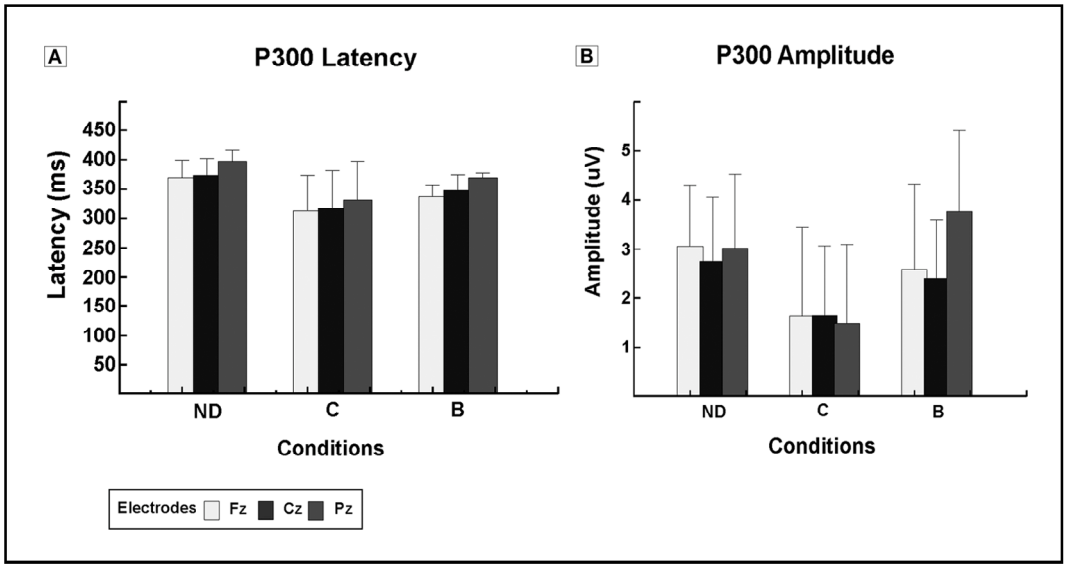


*Fig 2. P300 latency (A) and amplitude (B) variations across conditions (ND, C, B) and electrodes (Fz, Cz, Pz).*

= 380.24 ± 28.72 ms; C = 320.42 ± 62.77 ms; B = 351.47 ± 23.21 ms. In relation to amplitude values, the two-way Anova revealed no interaction between condition and electrode site (p = 0.301). No main electrode effect was found (p = 0.303). However, there was a significant effect for condition (p = 0.000). The post hoc (Scheffé) indicated a difference between ND and C (p = 0.000) and between C and B (p = 0.000). Mean amplitude values for each condition were: ND = 2.95 ±1.33 μV; C = 1.58 ± 1.61 μV; B = 2.93 ± 1.62 μV.

## DISCUSSION

The present study compared the neuromodulatory effects of caffeine and bromazepam, in relation to the P300 normative database, on the visual event-related potential. The study design was established to compare experimental conditions and different cortical areas (two-way Anova). Motor and electrophysiological responses were observed. Thus, the following discussion will be divided into three dependent variables used to compare the effects of drug intervention: a) Reaction time, b) P300 latency, and C) P300 amplitude.

*Reaction time* – Reaction time was assessed to analyze stimulus recognition, motor response and sensorimotor performance. Specifically, reaction time is the amount of time it takes a person to process an environmental (i.e., internal or external) signal. Some studies have analyzed the effects of drugs caffeine and bromazepam on reaction time. Kawamura et al.[13] observed that, depending on stimulus modality (oddball or single tone), P300 amplitude and reaction time would yield different results when 500 mg of caffeine was administered . In our study, subjects were rested and the effects of caffeine in behavioral aspects seem to be more evident in a fatigue situation[28]. In addition, behavioral responses are closely related to the biological state (i.e., alertness, fatigue, motivation) of the individual. In this sense, with subjects in rest condition, the stimulants effects are diminished. Jansen et al. examined the effects of bromazepam (6 mg and 12 mg) on reaction time sixty-five minutes after drug administration, and observed decreased perform-ance [20]. Bourin et al. investigated the effects of bromazepam (3 mg) and other benzodiazepines on twenty healthy volunteers. Bromazepam effects were evaluated 2 and 6 hours after administration and no significant difference was shown 2 hours after drug ingestion. However, a longer motor reaction time was observed 6 hours after bromazepam ingestion[21]. In our study, the effects of caffeine (400 mg) and bromazepam (3 mg) were observed 30 minutes after drug ingestion, and the statistical analysis did not indicate any difference between the conditions when compared to the normative database. In other words, an expected lengthening of reaction time was not observed. It may be argued that the results may be influenced by the dosage administrated. It may also be possible that reaction time is not a sensitive measure to detect drug effects on the conditions described in the present experiment.

*P300 latency* – The present results showed no interaction between condition and electrode site. However, main effects for condition and electrode site were observed. Since caffeine is a CNS stimulant and bromazepam is a depressor, it was expected that the two experimental conditions would differ from the normative database. Studies have pointed out to an improvement of cognitive performance after caffeine ingestion, through a significant shortening of P300 latency, especially in the frontal cortex[12]. This cortical region is closely related to attentional demands. In this context, the expected pattern of results was observed; caffeine's mean latency was significantly shorter than the other two conditions, which may be explained by an increased release of neurotransmitters (e.g., noradrenaline, serotonin, and acetylcholine) and by caffeine's property as an adenosine antagonist. On the other hand, very few studies have combined bromazepam and ERPs, and the results of these experiments are controversial and not conclusive[23]. However, we expected to find longer latencies in the bromazepam condition due to the drug's GABA enhancement property. GABA is the major inhibitory neurotransmitter and benzodiazepines improve GABA activity in different CNS areas. In other words, we expected an impaired cognitive functioning after bromazepam administration. Although the bromazepam was significantly different from the two other conditions, i.e., caffeine and normative database, bromazepam's mean latency was shorter than the normative database's. Once again, the dosage used may account for this result. Another possible explanation may be that bromazepam acted primarily on the subjects' anxiety level. In this sense, after drug ingestion and a subsequent reduction of the anxiety level, subjects' ability to process sensory information improved. Thus, this particular pattern of results indicate a faster stimulus detection process. Finally, as stated previously, the electrode main effect was expected once the pattern of latency distribution across different electrodes occurs independently from the drug administration. In other words, P300 latency increases from the anterior to the posterior scalp areas, i.e., from Fz (frontal), to Cz (central), and Pz (parietal) electrode sites, independently from others variants[26].

*P300 amplitude* – In relation to amplitude values, there was also no interaction between condition and electrode site. In addition, no main electrode effect was observed. One possible explanation for this pattern of results is the fact that amplitude values have a great variability. However, a main condition effect was observed in the present study. Reeves et al. observed a shortening of P300 latency but no changes in P300 amplitude with 13 caffeine-user subjects, submitted to four days of caffeine-deprivation[12]. Recently, investigators observed hypoglycemia in normal[29] and diabetic[30] subjects through changes in P300 outcomes. In these studies, a decrease in amplitude and a delay in latency, in relation to low concentrations of plasmatic glucose, were observed. Thus, results of the effects of caffeine on P300 amplitude are still contradictory. We expected to observe a higher mean amplitude in the caffeine condition when compared to the other conditions, due to a possible enhancement of attentional levels. However, results show a lower amplitude value for the caffeine condition when compared to the normative database and bromazepam. One possible explanation is that caffeine appears to increase P300 amplitude only when subjects are fatigued[26]. The only difference that was not observed, as shown by the post hoc, was between normative database and bromazepam. Again, the dosage used in this study (3 mg) may not have been suffiecient to cause differences on cognitive processes assessed by this ERP measure.

The results of the present study indicate that caffeine and bromazepam have distinct effects on CNS functioning, as assessed by the P300. Such differences may be explained by the specific neurophysiological mechanisms of these two substances. Further studies, using different doses of bromazepam and caffeine are necessary to understand the effects of these two psychoactive substances on cognitive and motor performance, as well as in brain dynamics.

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