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THE EFFECTS OF BROMAZEPAM ON THE EARLY STAGE OF VISUAL INFORMATION PROCESSING (P100)

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ABSTRACT - The early stages of visual information processing, involving the detection and perception of simple visual stimuli, have been demonstrated to be sensitive to psychotropic agents. The present study investigated the effects of an acute dose of bromazepam (3 mg), compared with placebo, on the P100 component of the visual evoked potential and reaction time. The sample, consisting of 14 healthy subjects (6 male and 8 female), was submitted to a visual discrimination task, which employed the "oddball" paradigm. Results suggest that bromazepam (3 mg) impairs the initial stage of visual information processing, as observed by an increase in P100 latency.

KEY WORDS: bromazepam, visual evoked potential, P100.

Efeitos do bromazepam no estágio inicial do processamento de informação visual (P100)

RESUMO - Os estágios iniciais do processamento da informação visual, envolvendo a percepção e detecção de um estímulo visual simples, tem demonstrado serem sensíveis a agentes psicotrópicos. O presente estudo investigou os efeitos de uma dose aguda de bromazepam (3 mg), comparado com placebo, no componente P100 do potencial evocado visual e no tempo de reação. A mostra consistiu de 14 sujeitos sadios (6 homens e 8 mulheres), submetidos a uma tarefa de discriminação visual, a qual empregou o paradigma "oddball". Os resultados sugerem que o bromazepam (3 mg) prejudica o estágio inicial do processamento da informação visual, como observado pelo aumento da latência do P100.

PALAVRAS-CHAVE: bromazepam, potencial evocado visual, P100.

Benzodiazepines, such as bromazepam, have been therapeutically used due to their sedative, hypnotic, muscle relaxant, anxiolytic, and anticonvulsivant properties[1]. Their mechanism of action on the central nervous system (CNS) is believed to be related to the ability to enhance the activity of the gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter[2-4]. Studies employing EEG parameters have demonstrated that bromazepam impairs a variety of neuropsychological functions such as memory, attention, psychomotor activity, reaction time, and vigilance performance[5-8]. Specifically, event-related potentials (ERPs), such as the P100, N200, P300 and N400, have been widely employed in the evaluation of the distinct stages of information processing and in the identification of changes in neural activation yielded by distinct drugs[9-11].

The occipitally distributed P100 component, in particular, is associated with the nerve conduction velocity of visual inputs from the retina to the primary visual cortex and, therefore, is related to the early stages of information processing, especially those involving the detection of simple stimuli and perceptual categorization of different stimulus modalities[12]. In this sense, the P100 has been extensively employed to characterize sensory-perceptual processes, such as somatosensory awareneness, facial recognition, and visuospatial attention[13-15]. Given that early stages of information processing are sensitive to pharmacological agents[16,17], P100 analyses enable a meaningful understanding of how a specific substance influences CNS ability to process incoming information. However, despite the vast number of studies employing ERP measures to understand changes in neuronal in-

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formation processing induced by psychotropic drugs, few have combined the P100 component and bromazepam. In a previous study, Puga et al.[18] analyzed the effects of bromazepam (3 mg) on the P300 component of the visual ERP (latency and amplitude), and on behavioral measures (Stroop, digit span, and reaction time). However, bromazepam did not produce any evident effects on the measures analyzed.

In this context, the present study aimed at complementing this first study by investigating if the intake of the same dose of bromazepam would produce any changes on the initial stage of visual information processing, through the P100 component of the visual evoked potential (VEP).

METHOD

The sample consisted of 14 volunteers, 6 male and 8 female, with ages varying between 21 and 38 years (26±4 years). All subjects were healthy, and did not use medication or any psychoactive or psychotropic substance at the time of the test. In order to increase group homogeneity, only right-handed subjects were included in the sample. To assure that subjects did not have any physical or mental health impairment, and to identify and exclude from the experiment any subjects who could contaminate future results, all participants were evaluated by a neuropsychiatrist. A questionnaire was developed and administered at the beginning of each test session to identify possible ERP biological determinants, such as food intake, body temperature, fatigue, drugs, among others. Subjects signed a consent form, where the experimental condition was thoroughly described. The experiment was approved to the Psychiatric Institute's ethics committee.

Subjects received a capsule (bromazepam or glucose) on two separate days, under a randomized, double-blind, crossover study. The procedures were presented in the following routine: 1) First visual evoked potential; 2) Administration of a capsule (bromazepam 3 mg, or placebo); 3) The second visual evoked potential, 20 minutes after capsule ingestion; 4) The third visual evoked potential, 60 minutes after capsule ingestion.

Visual evoked potential: data acquisition and analysis – A sound-attenuated room was prepared for data acquisition. Subjects were seated comfortably in a chair with armrest 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 P100, all subjects were administered the same visual discrimination task, which employed the "oddball" paradigm. In this paradigm, two stimuli are presented randomly, with one occurring infrequently[19]. The subjects were asked to discriminate the target (infrequent) from the 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 mounted on a joystick (Model Quick Shot-Crystal CS4281) with their right index finger. Individuals' reaction time was measured at each trial. Each subject received one block of stimulus presentation. In each block, there was a 95% chance of 1 to 4 non-target stimuli preceding a target stimulus and a 5% chance of 5 to 7 non-target stimuli preceding a target stimulus. Specifically, 100 target stimuli were always presented in each block. The total number of stimuli presented, targets plus non-targets, varied between 350 and 400 in each block. The stimulus appeared on the screen for 0.75 seconds with and inter-trial interval (onset to onset) of 1.5 seconds.

The International 10/20 System[20] for electrode placement (referred to linked earlobes) was used with a 20-channel Braintech-3000 (EMSA-Medical Instruments, Brazil). The 20 monopolar electrodes were arranged in a nylon cap (Electro Cap 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 EEG data acquired had total amplitude of less than 100 μV. The signal was amplified with a gain of 22,000. 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[21]. The EEG signal was analog filtered between 0.16 Hz (high-pass) and 35 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 a 60 Hz *notch*.

Filter and epoch selection routines were used to process acquired digital data. After data were acquired and stored, the averaging software loaded the data and established different routines. The target stimulus (square) was selected as the trigger-stimulus. Epochs (i.e., visualization windows) were set to begin 120 ms pre-stimulus onset through 700 ms post-stimulus. After specific channels were selected (O1, Oz and O2), data were averaged and represented graphically in terms of latency (x-axis) and amplitude (y-axis). Epochs related to target stimuli were considered and averaged only when subjects responded between 150 and 1000 ms. The P100 component was identified as a positive component within the latency window of 50-150 ms. Amplitude was measured at peak latency and relative to a pre-stimulus baseline of 120 ms. Peak latency was defined as the time point of maximum positive amplitude within the specific latency window.

Reaction time – Volunteers responded to the target stimulus by pressing a button in a joystick. Although reaction time is not directly related to ERP measures, it was used to verify subjects' alertness during the task and as an index of individuals' motor performance in the oddball task. The joystick was used to measure individuals' reaction time at each trial. Missed stimuli were not considered.

Statistical analysis – For reaction time, a two-way ANOVA, drug condition x time point (2 x 3), was applied. For the electrophysiological variables, latency and amplitude (target P100), a three-way ANOVA, drug condition x time point x electrode (2 x 3 x 3) was performed. A post hoc (Scheffé) was applied a posteriori. The experimental drug conditions were defined as: bromazepam and placebo. Experimental time points were 0′ (baseline), 20′, and 60′ after capsule ingestion. The electrodes analyzed were O1, Oz, and O2.

RESULTS

Behavioral – Figure 1 expresses the variation in mean reaction time (oddball task) across the two established drug conditions (bromazepam and placebo) and time points (0′, 20′, 60′). The reaction time variable refers exclusively to the correctly detected targets. No main time point effect F (2, 81)=0.41; p= 0.661 or drug condition effect F (1, 82)=0.09; p=0.927 were found. No interaction was observed F (2, 168)= 0.845; p=0.434.

Electrophysiological – Figure 2 illustrates P100 latency variations across drug conditions, and time points, at the O1, Oz, and O2 electrode sites. The three-way ANOVA revealed a main effect of drug condition F (1, 250)=23.87; p=0.000. Specifically, a significant increase in latency was observed in the bromazepam condition when compared to the placebo. The analysis also revealed main effect of time point F (2, 249)=3.38; p=0.036, characterized by an increase across time points. The post hoc analysis indicated a significant difference between 0′ and 60′ p=0.037. No main effect of electrode was found F (2, 249)=0.03; p=0.969. The analysis also pointed out to an interaction between drug condition and time point F (2, 504)=18.65; p=0.000. In this sense, results show a constant increase in latency values across time points, which was more evident in the bromazepam group.

Figure 3 illustrates P100 amplitude variations across the same drug conditions, time points, and

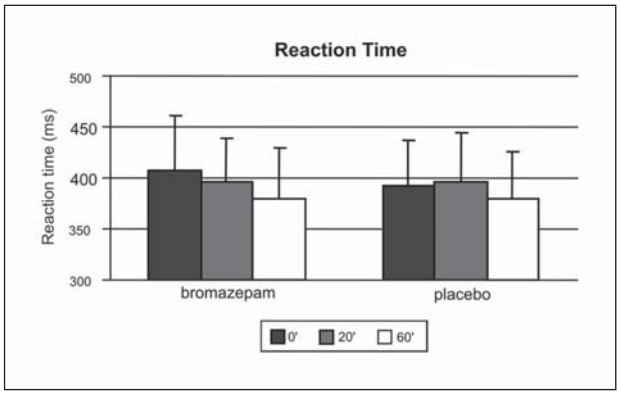

Fig 1. Reaction time mean score variation across experimental drug conditions (bromazepam and placebo) and time points (0′, 20′, and 60′).

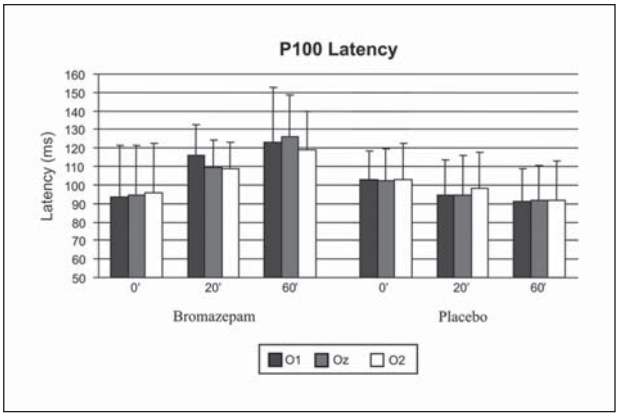

Fig 2. P100 latency (mean / std. error) across drug conditions (bromazepam and placebo) and time points (0′, 20′, and 60′), in O1, Oz, O2 electrode sites.

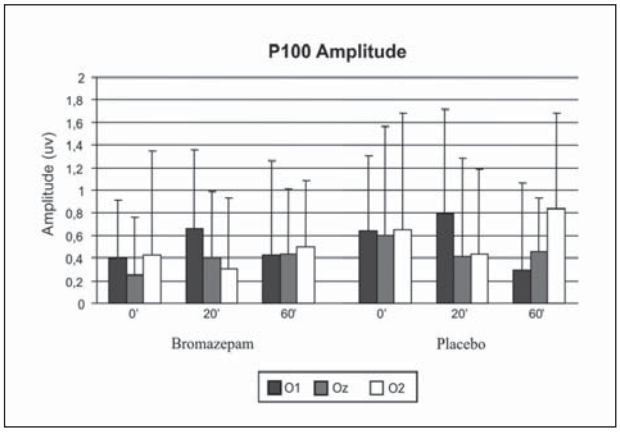

Fig 3 – P100 amplitude (mean / std. error) across drug conditions (bromazepam and placebo) and time points (0′, 20′, and 60′), in O1, Oz, O2 electrode sites.

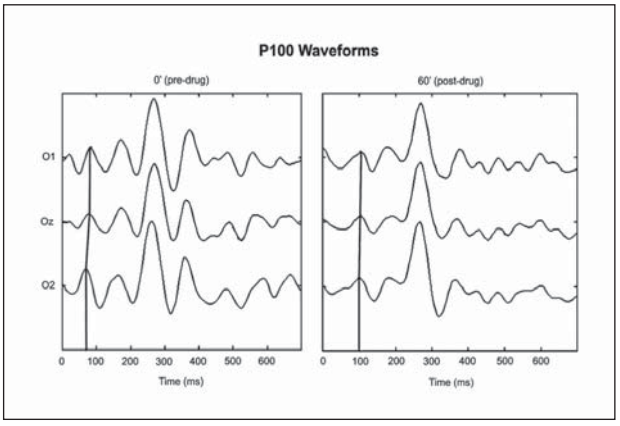

Fig 4. P100 waveforms at O1, Oz and O2 electrode sites and at 0′ and 60′time points.

electrode sites. The ANOVA revealed no main effects of drug condition F (1, 250)=2.44; p=0.120, time point F (2, 249)=0.00; p=0.997, or electrode site F (2, 249)= 0.50; p=0.605. No interactions were observed. Even though the differences were not statistically significant, P100 amplitude tended to be smaller in the bromazepam condition when compared to the placebo.

Figure 4 shows P100 waveforms at different electrode sites and at pre and post-drug time-points (0' and 60'), where latency differences were statistically significant.

DISCUSSION

The present study aimed at investigating the effects of bromazepam (3 mg) through behavioral and electrophysiological variables. The results were compared among conditions (placebo and bromazepam), time points (before, 20 and 60 minutes after drug intake) and cortical areas (O1, Oz, and O2). The following discussion will be divided into two dependent variables, which were used to assess the effects of drug intervention: a) reaction time and b) P100 (latency and amplitude).

Reaction time – Reaction time was used to verify subjects' alertness during the task and to analyze stimulus recognition, motor response, and sensorimotor performance. In the present study, the effects of a single oral dose of bromazepam (3 mg) and placebo were observed before, 20' and 60' after drug ingestion. The statistical analysis did not indicate any drug condition or time point main effects. Some studies have analyzed the effects of bromazepam on reaction time. 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 performance[6]. 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[7]. In another study, Hobi et al. observed a lengthening of reaction time for all three groups (placebo, bromazepam 1.5mg and bromazepam 3mg), but concluded that this result was due to the type of experimental design used, and only slightly to the drug's action[22].

Considering the results of the studies cited above, it can be concluded that the results may be influenced by the methodological factors such as the dosage administrated, the task employed, and time after drug administration that reaction time was measured. It may be possible that reaction time is not a sensitive measure to detect drug effects on the conditions described in the experiment.

P100 – It is speculated that detriments to the P100 might compromise other components (P300 and N400, for example), either by decreasing amplitudes and/or increasing latency values. Thus, the integrity of information processing seems to be dependent on the reliability of early visual inputs[23]. Given that impairment of these inputs may underlie the failure of high-level processes, such as attention, memory and sensorimotor performance, and once the P100 has been demonstrated to be sensitive to pharmacological agents, the understanding of the effects of specific drugs on the initial stages of information processing becomes imperative.

To elucidate this issue, the present experiment addressed the modulatory effects of bromazepam on the P100 using the "oddball" paradigm. We expected to find longer latencies and lower amplitudes in the bromazepam condition due to the drug's GABA enhancement property. In other words, we expected to see an impairment of the early stage of information processing after drug intake. The results partially confirmed this hypothesis. A noted increase in latency values was observed after bromazepam ingestion, showing that a single oral dose of bromazepam (3 mg) can modify the time of synaptic conduction on this early stage of visual information processing. Therefore, the statistically significant increase in P100 latency values across time points in the bromazepam group can be understood as a result of the GABAergic effect of the drug, which increases the inhibitory postsynaptic potential (IPSP) on the visual cortex.

However, in relation to amplitude values, only a trend of decrease was seen. The absence of statistically significant differences for amplitude values might have been caused by other factors, such as the dosage employed, methodological aspects and the anxiety level of the subjects. Hence, a single bromazepam dose of 3 mg seems to directly interfere on the speed of information processing (latency), but not on the allocation of attentional resources during the given task (amplitude).

As stated previously, few studies have associated P100 variability with the administration of benzodiazepines. Recently, Pompéia et al.[17] investigated specific changes in visual perception produced by two different substances. They observed that P100 latency increased after lorazepam (2.0 mg) and flunitrazepam

(1.2 mg) intake when compared to a placebo group. No significant changes were observed for P100 amplitude. In another study, van Leeuwen et al.[24] evaluated the effects of two acute doses of oxazepam (20 and 40 mg) on the vigilance performance of 18 male subjects. The amplitudes of distinct PEV components (P1, N1, P2N2 and P3) were analyzed. Results indicated that the drug reduced the amplitudes of all ERP waves. The authors concluded that oxazepam impairs all aspects of information processing, as manifested in the various ERP waves, suggesting a state of general sedation. The authors also argued that the P100 may, to some extent, be considered endogenous once effects of task manipulation and attention were observed in a time window between 28 and 100 ms. Rockstroh et al.[16], in a different study, analyzed the effects of gradually increasing doses of clonazepam on 36 male volunteers submitted to a VEP task using a checkerboard reversal procedure. P100 amplitude reduced in the clonazepam condition when compared to the placebo. Latency did not significantly differentiate treatment groups. In this context, the results of the present study are in accordance with the literature. It appears that performance impairment produced by a benzodiazepine may be reflected by alteration either in amplitude values, latency or both. It must be stressed that the different methodologies used in these studies may account for the different results reached by each group.

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