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Effects of bromazepam in frontal theta activity on the performance of a sensorimotor integration task: A quantitative electroencephalography study

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ABSTRACT

Our objective is to verify the modulatory effects of bromazepam on EEG theta absolute power when subjects were submitted to a visuomotor task (i.e., car driver task). Sample was composed of 14 students (9 males and 5 females), right handed, with ages varying between 23 and 42 years (mean = 32.5 ± 9.5), absence of mental or physical impairments, no psychoactive or psychotropic substance use and no neuromuscular disorders (screened by a clinical examination). The results showed an interaction between condition and electrodes ($p = 0.034$) in favor of F8 electrode compared with F7 in both experimental conditions (t-test; $p = 0.001$). Additionally, main effects were observed for condition ($p = 0.001$), period ($p = 0.001$) and electrodes ($p = 0.031$) in favor of F4 electrode compared with F3. In conclusion, Br 6 mg of bromazepam may interfere in sensorimotor processes in the task performance in an unpredictable scenario allowing that certain visuospatial factors were predominant. Therefore, the results may reflect that bromazepam effects influence the performance of the involved areas because of the acquisition and integration of sensory stimuli processes until the development of a motor behavior based on the same stimuli.

The investigation of cortical activity is essential for the understanding of the neural mechanisms related to psychoactive substances. In this context, electroencephalography (EEG) has been used to monitor the effects of distinct medications on brain dynamics since cortical activity is responsive to the unique characteristics of psychoactive substances [10,20]. The EEG sensitivity in identifying changes produced by a specific substance may be improved by methods of quantitative analyses (qEEG) [12]. Electroencephalographic data acquired through event-related potentials (ERPs) point out to the effects of this drug on the initial stages of information processing, making the individual gather less information from the sensory signals for a final evaluation [17]. Once drugs have specific effects on wave morphology, changes in qEEG variables can be used to investigate mechanisms of drug action as well as to monitor and possibly predict efficacy [7,21].

Cunha et al. [4] aimed to analyze the effects of bromazepam (Br 3 mg and 6 mg) on qEEG variables. It was found that the anxiolytic effect intensifies the attentional focus over predictable events occurring in reduced perceptual fields. The accentuated response in the premotor and primary motor areas suggests a higher effort directed to the most relevant aspects of the task. The results suggested an enhancement of the typewriting motor learning due to improvement the focused attention related to effects of the Br 6 mg.

In this manner, our objective is to verify the modulatory effects of bromazepam on EEG theta absolute power when subjects were submitted to a visuomotor task (i.e., car driver task). The increase of theta power has been related to increase in mental effort during the encoding of sensory information, attentional demand, higher task difficulty and increasing cognitive load [15,16,27]. Our task involves a certain degree of unpredictably and a decision-making process. According to these principles and considering the features of each cerebral hemisphere, our hypothesis is that through theta absolute power it would be possible to investigate the effects of bromazepam in specific cortical areas. We expect to see different theta absolute power patterns among the experimental and placebo groups in the

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frontal areas due to drug's influence at attention, action execution and sensorimotor integration processes.

Sample was composed of 14 students (9 males and 5 females), right handed [19], with ages varying between 23 and 42 years (mean = 32.5 ± 9.5). Inclusion criteria were absence of mental or physical impairments, no history of psychoactive or psychotropic substance use and no neuromuscular disorders (screened by a clinical examination). All subjects signed a consent form and were aware of all experimental protocol. The experiment was approved by the Ethics Committee of Federal University of Rio de Janeiro (IPUB/UFRJ). This experimental paradigm has been already used in other experiment [9].

The task was performed in a sound and light-attenuated room, to minimize sensory interference. All subjects were exposed to 3 experimental conditions, thus, the subjects were exposed to placebo (PL), bromazepam 3 mg (Br 3 mg) or bromazepam 6 mg (Br 6 mg), respecting a randomized double-blind design on different days. After the capsule ingestion, subjects remain at rest for 1 h. Then, a computer monitor (Sansung-SyncMaster 550 v) was positioned ahead of the subjects, sat on a comfortable chair to minimize muscular artifacts while electroencephalography (EEG) data was recorded before, during and after the motor task execution.

To accomplish the task, the subjects performed a visuomotor task (S1–S2 paradigm—car driver). The task was controlled and synchronized with qEEG recording by software Car Acquisition. The visuomotor task consisted of to drive a car in slow and fixed velocity paying attention on the curves, and to respond as quickly possible, when the action command appeared. The subjects should respond by pressing the anterior bottom of the *joystick* (Model Quick Shot-Crystal CS4281) fixed on a support under chair, in order to avoid the hand instability. Each subject was submitted to 50 trials in each experimental condition. The task was composed of 0.5 ms periods, before and after the appearance of each stimulus (i.e., pre-S1, post-S1, pre-S2 and post-S2). The warning stimulus (S1—yellow squad) and imperative stimulus or action command (S2—red triangle) appeared at a fixed interval of 2.5 s among themselves (intrastimulus interval). However, the interval between the appearance of S2 and S1 varied randomly among 2.5 and 15 s (interstimulus interval) with objective of do not offer cues in relation to occurrence of S1.

EEG—the International 10/20 System for electrodes [13] was used with the 20-channel EEG system Braintech-3000 (EMSA-Medical Instruments, Brazil). The 20 electrodes were arranged in a nylon cap (ElectroCap Inc., Fairfax, VA, USA) yielding monopolar derivations referred to linked earlobes. In addition, two 9 mm diameter electrodes were attached above and on the external corner of the right eye, in a bipolar electrode montage, for eye-movement (EOG) artifacts monitoring. Impedance of EEG and EOG electrodes was kept between 5 and 10 kΩ. The data acquired had total amplitude of less than 100 μV. The EEG signal was amplified with a gain of 22,000, analogically filtered between 0.01 Hz (high-pass) and 100 Hz (low-pass), and sampled at 240 Hz. The software Car Acquisition (Delphi 5.0) at the Brain Mapping and Sensory Motor Integration Lab, was employed with the following digital filters: notch (60 Hz), high-pass of 0.3 Hz and low-pass of 25 Hz.

To quantify reference-free data, a visual inspection and independent component analysis (ICA) were applied to remove possible sources of artifacts produced by the task. A classic estimator was applied for the power spectral density (PSD), or directly from the square modulus of the FT (Fourier Transform), which was performed by MATLAB 5.3 (Matworks, Inc.). Quantitative EEG parameters were extracted from 2 s periods (the selected epoch started 0.5 ms before and after the appearance of each stimulus, i.e., S1 and S2, respectively), for consecutive (non-overlapping) artifact-free, 2-s EEG epochs (spectral resolution: 0.25 Hz), with rectangular windowing. In this manner, based on artifact-free EEG epochs, the threshold was defined by mean plus three standard deviations and epochs with total power higher than this threshold were not integrated in the analysis.

We analyzed the electrodes F3, F4, F7, F8, C3 and C4. The F3 and F4 electrodes represent the premotor cortex, functionally responsible for preparing and voluntary control of action [23]. The F7 and F8 electrodes represent the prefrontal cortex, functionally responsible for executive functions [25]. The C3 and C4 electrodes are placed on the pre-central and central gyri, which are located in the primary motor areas. These areas are functionally related to motor execution [24]. In relation to theta band (4–8 Hz), it was chosen due to its association with cognitive functions such as, encoding of sensory information [2], attentional mechanisms [23] and information transmission [14].

The qEEG absolute power values were $\log_{10}$-transformed by SPSS software (version 16.0) to approximate a normal distribution. A three-way ANOVA and a Scheffe post hoc test were used to analyze the factors condition (i.e., PL × Br 3 mg × Br 6 mg), period (i.e., pre-S1, post-S1, pre-S2, post-S2) and electrodes (i.e., F3 vs. F4, F7 vs. F8, C3 vs. C4). We use a *t*-test ($p \leq 0.05$) to verify difference in the factors, just in case it was found some interaction.

The statistical analysis demonstrated an interaction between the factors condition and electrodes ($p = 0.034$) when compared with F7 and F8 electrodes. Moreover, a main effect for factors condition ($p = 0.001$), period ($p = 0.001$) and electrodes ($p = 0.031$) were found when compared with F3 and F4 electrodes. The examination of the interaction between condition and electrodes factors showed a significant difference in Br 6 mg and Br 3 mg conditions (*t*-test; $p = 0.001$). In both results, a higher absolute power in favor of F8 electrodes was observed. No significant difference was observed in PL condition, as observed in Fig. 1.

Moreover, a condition main effect was found when compared with the F3 and F4 electrodes. Scheffé analysis showed that Br 6 mg condition was significantly lower when compared with PL and Br 3 mg conditions in terms of absolute power values ($p = 0.001$). No significant difference was found among PL and Br 3 mg conditions (see Fig. 2). In the same way, a period main effect was found. It was observed that the post-S1 and -S2 periods demonstrated a significant increase in absolute power values when compared with pre-S1 and -S2 periods (Scheffé test; $p = 0.001$), as indicated in Fig. 3. No significant difference was found between the post-S1 and -S2 periods, as well as pre-S1 and -S2 periods. Lastly, in relation to electrodes main effect, a significant difference between the F3 and F4 electrodes ($p = 0.031$) was observed. A higher absolute power values in favor of F4 electrode was noted (see Fig. 4).

Our objective is to verify the modulatory effects of bromazepam on qEEG theta absolute power when subjects were submitted to a visuomotor task.

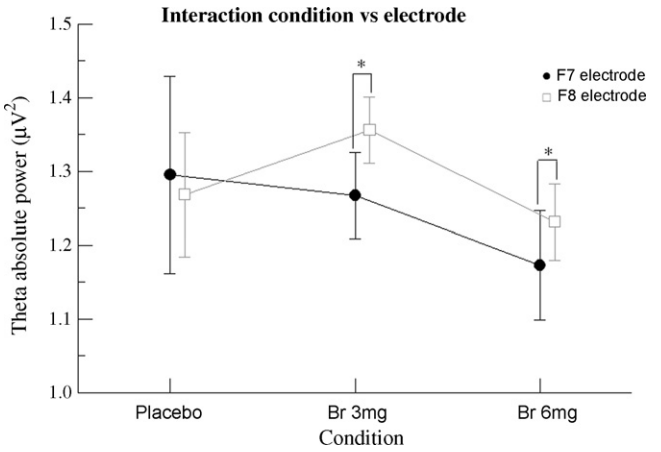

Fig. 1. Interaction between condition and electrode factors observed through mean and standard deviation values. *Significant difference (*t*-test; $p < 0.001$).

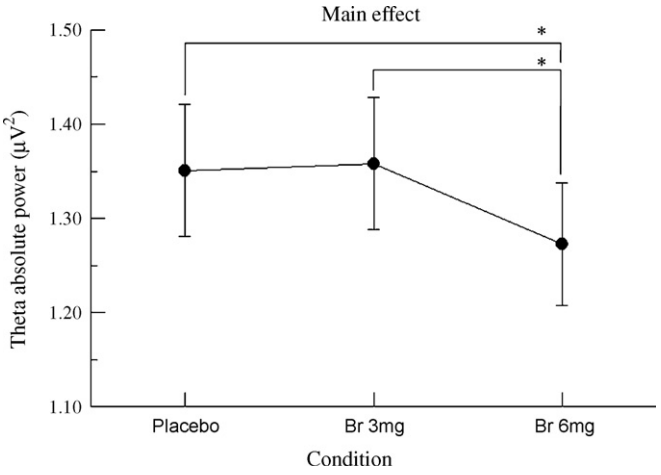

Fig. 2. Main effect for factor condition observed through mean and standard deviation values. *Significant difference, $p < 0.001$.

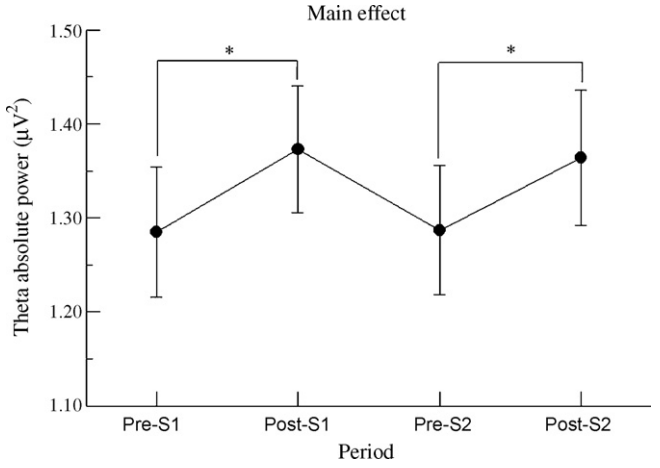

Fig. 3. Main effect for factor period observed through mean and standard deviation values. *Significant difference, $p < 0.001$.

We observed an interaction between condition and electrodes. It suggests that condition and electrodes would better explain theta power expression instead of the main effects isolated. In this manner, a significant difference (t-test) in Br 6 mg and Br 3 mg conditions was observed. In both results, a higher absolute power values in favor of F8 electrode was noted. There are several interpretations related to theta activity, depending on type of experimental task and involved cortical region. The increased theta power has been relating to increased mental effort during the encoding of sensory information, attentional demand, higher task difficulty and increasing cognitive load [15,16,27]. In this manner, the increased theta power found in F8 electrode in Br 6 mg compared with Br 3 mg conditions can be interpreted as a reduction in overload mental functions. Besides, it is acknowledged that there is a functional difference between the left and right cerebral hemispheres. It is acknowledged that right hemisphere owns a narrow relationship with visuospatial functions; in contrast to left hemisphere that has a dominant role in the control of movement functions [22]. Consistent with other studies, in which observed a higher theta power in an unpredictable scenario [14], our results suggested a higher involvement of the right prefrontal area in processes as, operational and spatial working memory and spatial attention. Operational memory works as temporary information storing to guide future actions. It is a form of motor planning and refers to active maintenance of relevant information for a behavior that is occurring. This fact supports our interpretation that right prefrontal cortex contributes to executive functions more powerfully, which are inherent elements of the decision-making process.

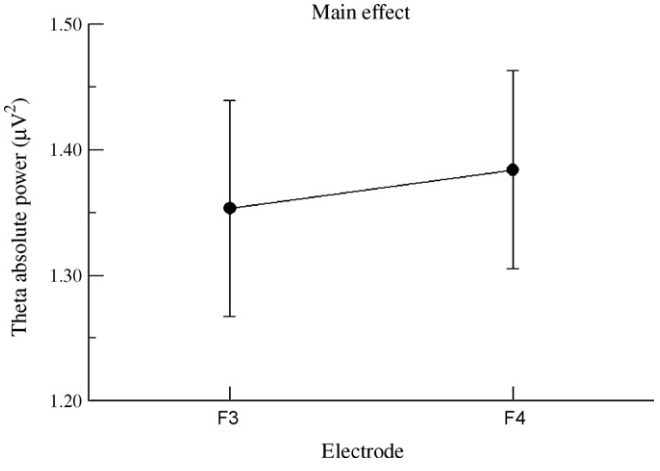

Fig. 4. Main effect for factor electrode observed through mean and standard deviation values.

The experimental task involves a relationship between a controlled object (i.e., a car) and a virtual environment (i.e., a track). According to our results, the subjects probably employed attentional mechanisms and spatial working memory to better perform the task. Actually, the subjects appear to have employed a conscious mental effort to store and recover route information (i.e., different forms and types of curve). Similar results were found in Caplan's experiment. According to Caplan et al. [3], increased theta power values were observed when healthy participants executed a goal-seeking visuomotor task (virtual task). More specifically, this increase was noted in right prefrontal area. Such fact was associated with states of focused concentration, and its enhancement might reflect the conscious control over attention associated with the maintenance of a task-appropriate mental set. In this sense, our findings could be interpreted as an attentional and spatial working memory interaction to better regulate the motor behavior. In relation to significant difference in favor of F8 electrode in Br 6 mg condition and the type of the task suggests an improvement of mental functions as, sustained attention and spatial working memory. In this sense, our results suggest that anxiolytic effects can improve the visuospatial-related processes over relevant aspects of the task [4], with less exposure to peripheral stimuli interference.

In relation to condition main effect, it was observed that Br 6 mg condition was significantly lower when compared with PL and Br 3 mg conditions in terms of absolute power values. This increased theta power found on Br 6 mg can be interpreted as a reduction in mental functions. In this manner, our results can be interpreted as the influence of drug effects on the task, which probably allowed motor preparation and voluntary control of action. In this sense, our results suggest that anxiolytic effects can improve attention over relevant motor aspects of the task [5,4]. Therefore, the CNS has less exposure to interference from internal mechanisms related to organization of parameters that will compose the motor scenario.

This experimental task involves unpredictable situations where a car is controlled in a virtual track. According to our results, the subjects exposed to Br 6 mg probably focused on attentional mechanisms more intensively for reducing the cognitive load that, in other ways, had received more interference through peripheral stimuli. Such effects seem reducing, in a certain degree, the complexity of the task (task difficulty). Additionally, the subjects appear to have encoded less sensory information to store and recover route information (i.e., different forms and types of curve). These findings were similar to Smith's results. According to Smith et al. [23],

increased theta power values were observed when healthy subjects performed a difficult visuomotor task (video game). Theta rhythm in frontal areas (Fz electrode) appeared to be increased over the course of the sessions. Such fact was associated with states of focused concentration, and its enhancement might reflect the conscious control over attention associated with maintenance of a task-appropriate mental set. Our interpretation is that there is an attention-related motor execution as a preparatory process for the preparation and voluntary control of movement to better regulate the motor behavior.

In relation to period main effect, it was noted that the post-S1 and -S2 periods demonstrated a gradually significant increase in absolute power values when compared with pre-S1 and -S2 periods. Actually, a theta absolute power peak in post-S1 and -S2 periods and a decrease in theta absolute power in pre-S1 and -S2 periods were observed. In this sense, the approximation of stimulus appearance (e.g., S1 and S2), it is suggested that the attentional demand progressively increases and the opposite behavior seems occurring after the stimulus identification. This theta reactivity does not seem to make distinction regarding to the stimulus, responding to warning and imperative stimuli similarly. Such fact probably means an increase in demand of theta-related processes after stimuli exposure. In our task the participants had to control a car in a virtual track. Additionally, the subjects had to encode sensory information to store and to recover route information (i.e., different forms and types of curve). The results are based on frequency domain, moreover, a few studies that had been used ERP support our main findings. These experiments demonstrated that the post-S1 and -S2 activities are believed to reflect the comparison between the stimulus input in progress and the pre-S1 and -S2 activities. The later stimuli are considered the sensory memory trace of the preceding stimulus [6,8].

Finally, in relation to electrodes main effect, a significant difference between the F3 and F4 was observed. A higher absolute power values in favor of F4 electrode was noted. In this manner, our results seem indicating that the right premotor cortex had a predominant role on the task execution. Therefore, the findings indicating that the right hemisphere is more activated due to several visuospatial features related to the experimental task. According to our results, it seems that several processes were requested by our experimental task. In this manner, several processes have been demonstrated as, involvement in the control of spatial attention for both the left and right visual fields [18] and monitoring function in conflict situations [26], when experiencing a mismatch between motor intention, proprioception and/or visual feedback [17]. Other important process is that, the involvement of right hemisphere to regulate and control posture and final limb position [1], due to crucial role on closed-loop aspects of the movement (dependent on sensory feedback) [11]. In this sense, our results suggest that when subjects performed the task, an increase in encoding of sensory information of mechanisms related to preparing and voluntary control of action occurred.

In conclusion, 6 mg of bromazepam may interfere in sensorimotor processes in the task performance in an unpredictable scenario allowing that certain visuospatial factors were predominant. Our task involves a decision-making and immediate response to unanticipated stimuli. Therefore, the results may reflect that bromazepam effects influence the performance of the involved areas because of the acquisition and integration of sensory stimuli processes until the development of a motor behavior based on the same stimuli. The present study has limitations the use of normal individuals as experimental subjects and the absence of a clinical evaluation of anxiety levels before and after the motor task.

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