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Neuromodulatory effect of bromazepam on motor learning: An electroencephalographic approach

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Abstract

To investigate the effects of bromazepam on motor performance and electroencephalographic activity (qEEG) in healthy subjects, during the process of learning a typewriting task, with a focused attention demand. A randomized double-blind model was used to allocate subjects in one of the following conditions: placebo ($n = 13$), bromazepam 3 mg ($n = 13$) or bromazepam 6 mg ($n = 13$). Forty minutes after treatment administration, subjects were submitted to the motor task. EEG activity was recorded simultaneously. The analyzed variables were: number of errors and execution time, which were extracted from each block of the typewriting task, and mean relative power values in the beta band (13–35 Hz), extracted from the qEEG. A significantly lower number of typing errors was observed in both bromazepam conditions (Br 3 mg and Br 6 mg) when compared to the placebo. There was no difference between the two bromazepam conditions. For the execution time variable, a better performance was observed in the Br 3 mg condition, but with no statistical significance. The highest degree of cortical activation during the task was observed in Br 3 mg and Br 6 mg when compared to placebo. The medication's anxiolytic effect intensifies the attentional focus over predictable events occurring in reduced perceptual fields. The qEEG's accentuated response in pre-motor and primary motor areas suggests a greater effort directed to the most relevant aspects of the task. In short, the doses employed (3 and 6 mg) seem to enhance the learning of motor tasks that involve focused attention, such as typewriting.

Bromazepam is a benzodiazepine that has been widely employed in the treatment of anxiety [4,6], despite the impairment of performance on psychometric tasks it produces. Electroencephalographic data acquired through event-related potentials (ERPs) points 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 [10]. Choice reaction time (CRT) results and the digit/symbol substitution test (DSST) scores suggest that bromazepam impairs the recognition

and processing of sensory data [2]. On the other hand, findings demonstrate that the muscle-relaxant and anxiolytic action of bromazepam exerts a positive effect on psychomotor function by way of stabilization or most likely by lessening nervous tension [4].

For such discussion it is important to consider bromazepam's cumulative effect on the body. In a study with normal subjects, bromazepam (1.5 and 3 mg) was administered during 14 days. Attention, alertness and reaction time tests were applied before, after 7 days, and after 14 days of administration. Results pointed out to a slowing of reaction time during the course of the study, especially for the 3 mg dose. This result was particularly evident after 7 days when compared to the other moments. Despite this observation, there was a significant training effect in the

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3 mg group with regard to attentiveness and alertness testing [5].

The present study investigated the effects of bromazepam during the motor learning of a typewriting task. This specific task involves a type of closed ability, with reduced perceptual field and a dynamic that enables the gain of automaticity [3,9,20]. The slight demand of peripheral sensory stimuli processing, if any, which is external to the individual, makes this task a useful tool to assess the influence of bromazepam on more inherent aspects of motor control. The qEEG data was explored in order to compare subjects' performance with a pattern of cortical activation induced by the task. To achieve this goal, changes in relative power in the beta band were analyzed, once this rhythmic activity is modulated by benzodiazepines [14] and is induced in sensorimotor areas during tasks that involve limb movements [1,17,18,21].

The sample was composed of 39 right-handed participants, according to the Edinburgh inventory [15]. The subjects were well informed of the aim of the study and voluntarily signed a free consent containing all experimental circumstances. The UFRJ Ethics Committee approved the experiment.

The subjects ingested capsules containing either placebo (glucose 400 mg), bromazepam 3 mg or bromazepam 6 mg. Tablet distribution followed a randomized double-blind design. Forty minutes after ingesting tablets, the typewriting task started concomitant with EEG recording, which lasted until the end of the task.

For the accomplishment of the task, an old model typewriter was chosen (Olivetti/Linea model 98). Subjects sat comfortably at a distance of approximately 20 cm from the typewriter. The typewriter keyboard was covered with a wooden box to avoid visual information about the hands' position. The task employed followed a typewriting method of progressive learning, in which training was performed on a single day. The exercise was made up of four blocks, each block represented by twelve lines. Each line had five sequences of letters for each hand. The established sequence of letters for each hand was: asdfg for the left hand, and clkjh for the right hand. When each sequence was over, space key was pressed using the left or right thumb (see Fig. 1).
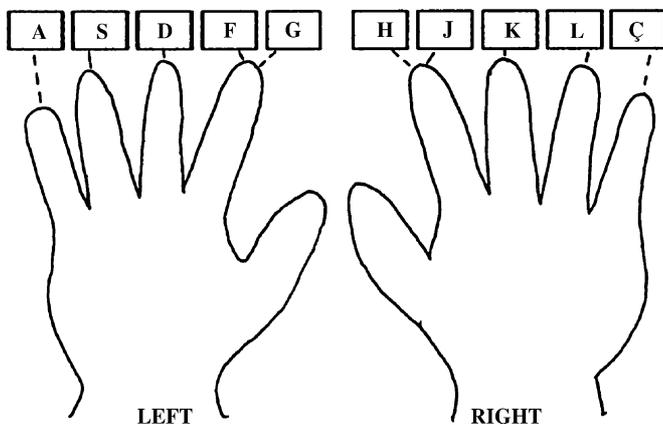

Fig. 1. Motor task of typewriting. Subjects pressed the marked keys "a", "s", "d", "f", "g" using their little, ring, middle and index finger of left hand and the keys "ç", "l", "k", "j", "h" using their little, ring, middle and index finger of right hand.

Below the typewriter, there was an electrical circuit responsible for the acquisition of letters h (i.e., right hand index finger). The circuit was made up of one U-shaped optoeletronic device. This had an emission and a receptor light segment, which conveyed the information regarding the pressing of the selected letter to the data acquisition system (the transfer was a simple D/A decoder).

The device Braintech 3000 (Emsa, Medical Instruments, Brazil) was used. This system uses an A/D converter plate of 32 channels with resolution of 12 bits, placed in a Pentium III ISA slot a notch with processor of 750 MHz. The electrophysiological signals were filtered between 0.01 (high-pass) and 100 Hz (low-pass), with a sampling ratio of 200 Hz. A software EEG Acquisition was used (Emsa-Delphi 5.0) with filter Notch of 60 Hz and cut-off filters of 0.3 Hz (high-pass) and 25 Hz (low-pass). The international 10/20 electrode system [7] was used for the placement of 20 monopolar electrodes along the scalp. The earlobes were used as reference (biauricular). The impedance was kept within the range of 5–10 kΩ. The signal was amplified with gains of about 20.000. The electroencephalographic signals obtained ranged from 0.01 to 50 Hz. Ocular artifacts were removed by visual inspection of EEG data with the aid of a visualization program (EEG Telas, Emsa-Delphi 5.0).

Quantitative EEG parameters were extracted from epochs from 2 s before to 2 s after movement onset (i.e., pressing h key). Automatic rejection (100 μV) and visual inspection were employed to avoid muscular and heart rate contamination of the data. Discrete Fourier Transform (DFT) was applied to extracted absolute power values on EEG bands (i.e., delta, theta, alpha and beta), respectively, before movement onset (i.e., between -2 and 0 s, labeled as Reference R) and after movement onset (i.e., between 0 and 2 s, labeled as Practice P) with Bartlett windows. The segment length for DFT was 2 s and there no overlapping between consecutive segments. Relative power was obtained from the ratio of total power for each frequency band. The h key in the typewriter machine was used to determine the zero seconds time of epochs. To facilitate visual inspection between R and P periods, a combination of low and high-pass filters (i.e., Butterworth second order) was applied. We averaged the numbers of h key pressing to estimate the temporal evolution of mean power (Matlab 5.3, MathWorks).

We grouped the electrodes in four scalp sectors: left-anterior (LA), composed by F3, C3 and T3, right-anterior (RA), including F4, C4 and T4, left-posterior (LP), which included T5, P3, O1 and right-posterior (RP), made of T6, P4 and O2. The two anterior sectors had their electrodes placed on the pre-central and central gyri, where the pre-motor and primary motor areas are located. These areas are functionally related to motor planning and motor execution [13,22]. The posterior sectors represent the primary somatosensory regions and association and visual areas.

For the analysis of the motor variables (i.e., time and error rates) on the typewriting task, a two-way ANOVA was employed, combining the factors: *condition* (i.e., placebo × Br 3 mg × Br 6 mg) and *blocks* (i.e., 1, 2, 3, 4 blocks). In the electrophysiological analysis, a three-way ANOVA was used to ana-

lyze the factors: *condition* (i.e., placebo × Br 3 mg × Br 6 mg), *moment* (i.e., 2 s before and 2 s after the letter h key pressing) and scalp *sector* (i.e., LA × RA × LP × RP). The EEG relative power values were log-transformed by *SPSS* software (version 10.0) to approximate a normal distribution. Since relative power is bounded between 0 and 1, the log values present negative results; hence an increase in negativity must be considered as a decrease in relative power. The significance level was considered $p \leq 0.05$.

A main effect of *condition* was observed ($p = 0.008$). The post-hoc analysis indicated a reduction in the number of errors in the Br 3 mg (mean = 71.2; S.D. = 35.9) and Br 6 mg (mean = 73.2; S.D. = 48.6) conditions when compared to the placebo (mean = 96.5; S.D. = 55.1). No significant difference was observed between Br 3 mg and Br 6 mg (see Fig. 2a).

Still for *number of errors*, a main effect of *block* ($p = 0.000$) was also observed. Post-hoc analyses showed that number of errors was reduced throughout the blocks during the task. However, these reductions were only statistically significant between the first block (mean = 108.6; S.D. = 60.5) and the third (mean = 66.1; S.D. = 41.2), and between the first and the fourth (mean = 66.6; S.D. = 36.2).

For the other motor variable, *execution time*, a *condition* main effect trend was observed, particularly between placebo and Br 3 mg ($p = 0.059$) (see Fig. 2b). In counterpart, a main effect of *block* ($p = 0.000$) was observed. Post-hoc analyses showed that execution time reduced throughout the blocks. Statistical significance was reached between the first block (mean = 517.4; S.D. = 157.8) and the second (mean = 419.9; S.D. = 96.5), between the first and the third (mean = 382.7;

Table 1
Summary of two-way ANOVA for error and time variables in typewriting task; *F* values and probabilities are presented

	Error		Time	
	F	*p*	*F*	*p*
Condition (C)	5.03	0.008	2.98	0.054
Block (B)	7.59	0.000	15.57	0.000
C × B	0.12	n.s.	0.57	n.s.

S.D. = 82.6), and between the first and the fourth (mean = 364.9; S.D. = 76.1). The results of the two-way ANOVA for error and time variables are summarized in Table 1.

In relation to the electrophysiological variable *relative power*, a *condition* main effect was observed ($p = 0.000$). Subsequent post-hoc analyses demonstrated more negative values (log) in Br 6 mg (mean = −0.232; S.D. = 0.035) and Br 3 mg (mean = −0.228; S.D. = 0.040) when compared to the placebo condition (mean = −0.209; S.D. = 0.039). Difference between the two bromazepam conditions was not statistically significant (see Fig. 3).

A *moment* main effect was also observed ($p = 0.039$). All the subjects showed a greater negativity of values in the post-movement moment (mean = −0.225; S.D. = 0.039) when compared to the pre-movement moment (mean = −0.221; S.D. = 0.040).

The last main effect refers to the factor *sector of the scalp* ($p = 0.000$). The post-hoc indicated a greater negativity of values in the anterior sectors, LA (mean = −0.243; S.D. = 0.028) and RA (mean = −0.233; S.D. = 0.033), when compared to the posterior sectors, LP (mean = −0.214; S.D. = 0.040) and RP (mean = −0.202; S.D. = 0.043). The result of the three-way ANOVA for relative power is presented in Table 2.

An enhanced motor performance in the two conditions (Br 3 mg e Br 6 mg) may be associated with a positive effect on psychomotor function by way of stabilization or by lessening nervous tension as suggested by another study [4]. Such benefit may also be associated with the nature of the task. The drug's positive effect occurs mainly in tasks where decision-making processes and immediate reactions to sensory stimuli are not crit-
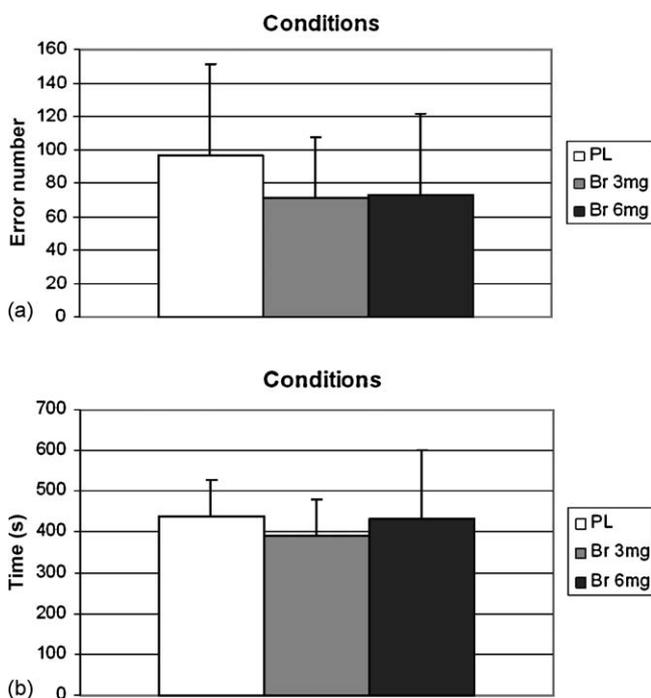

Fig. 2. (a) Error number in the typewriting task for each condition (PL, Br 3 mg and Br 6 mg). A main effect was observed. (b) Execution time in the typewriting task for each condition (PL, Br 3 mg and Br 6 mg). No differences were found.
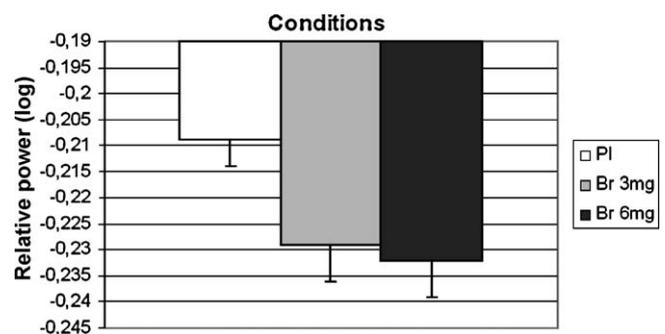

Fig. 3. Comparison of relative power in beta frequency between conditions: PL, Br 3 mg and Br 6 mg. A main effect was observed when we compared PL and Br 3 mg or PL and Br 6 mg.

Table 2
Summary of three-way ANOVA in beta frequency band; *F* values end probabilities are presented

	Beta	
	F	*p*
Condition (C)	40.95	0.000
Moment (M)	4.29	0.039
Sector (S)	64.79	0.000
C × M	1.24	n.s.
C × S	0.54	n.s.
M × S	0.45	n.s.
C × M × S	0.04	n.s.

ical for performance. This observation is in agreement with the results of other studies that suggested that bromazepam would have a negative effect in initial stages of sensory processing [2,10]. The learning of the typewriting task directs the individual towards a pre-programmed execution of the movement sequence [9,20], not taking into account several initial stages of information processing that would be hindered by bromazepam.

The increased levels of negativity (activation) in the beta band, which were observed in the Br 3 mg and Br 6 mg conditions when compared to placebo, suggest that the medication favored a higher concentration of electrocortical activity over somatomotor functions [12,18] that are relevant to the task. Such concentration could have been enhanced due to a decrease of activity related to the processing of sensory stimuli in the exterior environment. In fact, this pattern of "focal activation/surround de-activation" is referred to in other studies as a typical brain mechanism [13,14]. In this case, bromazepam must have favored the occurrence of the referred process even more.

The highest level of negativity was observed 2 s after the movement onset when compared to 2 s before, and as demonstrated in the three conditions, it confirms the results of other laboratories, which have reported a higher activation during the motor act when compared to the activation during motor preparation [8,19]. Our results indicate that bromazepam does not change this activation pattern, as demonstrated by the lack of an interaction between condition and moment.

The increased levels of negativity observed during the task occurred in the LA and RA sectors, indicating a greater usage of these regions for the execution of the motor act. This spatial activation pattern is in accordance with the literature [11,16,21], since these sectors encompass pre-motor and primary motor areas. Such areas are involved in the planning and execution of movements of contralateral limbs and bromazepam did not alter this spatial activation pattern.

In conclusion, the doses employed in the study may facilitate motor performance in tasks involving focused attention and executed in a stable scenario (closed ability). Tasks involving decision-making and immediate response to unanticipated stimuli may be more impaired by bromazepam and, thus, need to be studied systematically. The present study presents as 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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