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Responsiveness of sensorimotor cortex during pharmacological intervention with bromazepam

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ABSTRACT

The aim of this study was to investigate the influence of bromazepam on EEG and the motor learning process when healthy subjects were submitted to a typewriting task. We investigated bromazepam due to its abuse by various populations and its prevalent clinical use among older individuals which are more sensitive to the negative effects of long half-life benzodiazepines. A randomized double-blind design was used with subjects divided into three groups: placebo ($n = 13$), bromazepam 3 mg ($n = 13$) and bromazepam 6 mg ($n = 13$). EEG data comprising theta, alpha and beta bands was recorded before, during and after the motor task. Our results showed a lower relative power value in the theta band in the Br 6 mg group when compared with PL. We also observed a reduction in relative power in the beta band in the Br 3 mg and Br 6 mg when compared with PL group. These findings suggest that Br can contribute to a reduced working memory load in areas related to attention processes. On the other hand, it produces a higher cortical activation in areas associated with sensory integration. Such areas are responsible for accomplishing the motor learning task. The results are an example of the usefulness of integrating electrophysiological data, sensorimotor activity and a pharmacological approach to aid in our understanding of cerebral changes produced by external agents.

Quantitative electroencephalography (qEEG) is a useful tool to explore possible changes in the cortical activity that occurs during motor learning. Different groups using qEEG have shown that the acquisition of new motor patterns produce changes in the power values in different frequency bands [6,11,19,20]. Theta rhythm can be induced through different types of manipulations and task attributes. Topographic patterns may be unique and mapped into particular tasks. Theta band has been correlated with cognitive functions such as: stimulus' encoding [4], attentional mechanisms [20], object's recognition [7], among others. Similarly, the alpha rhythm behavior has been associated with various processes, such as visual perception [20], energetic demands [15,22] and motor functions organized somatotopically on the primary motor cortex [15]. With respect to the beta rhythm, it has been suggested to be closely related to sensorimotor propagation [1]. Moreover, EEG has been proved to be sensitive to pharmacologic manipulations of the central nervous system (CNS). Several studies have demonstrated an increase of the beta rhythm over diffuse cerebral cortical areas when individuals were exposed to benzodiazepines [16]. Some experiments have explored other effects of benzodiazepines on the CNS, combining qEEG, benzodiazepine use and procedural learning measures. Benzodiazepines became the worldwide most prescribed and abused pharmacologic group in the management of anxiety and insomnia [14]. However, most of the prescriptions have been made by non-psychiatrist physicians [2], and its influence on motor learning processes is not entirely understood. Some studies have shown that bromazepam may impair psychomotor capacity when individuals are submitted to neuropsychological testing [5,9,10,13]. It has been suggested that the impairment caused by bromazepam takes place on the early stages of sensorimotor integration, such as, stimulus detection and attention [13]. On the

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other hand, other studies suggest that low dosages of bromazepam improve cognitive and motor performance [6,8]. In this study, the authors suggested that the positive effect of bromazepam may be related to a control of anxiety with a reduction of internal tension [8]. From a different perspective, experimental results have suggested that bromazepam enhance the action of GABA at the GABA-A receptor, which are spread throughout the whole CNS. The objective of the present study was to investigate the influence of bromazepam on the motor learning process by qEEG when subjects were submitted to a typewriting task. In particular, we observed relative power measures in the theta, alpha and beta bands.

The sample comprised 39 healthy subjects (20 men and 19 women, aging between 20 and 30 years old; mean = 26.2, S.D. = 4.6), right-handed according to the Edinburgh inventory [17] was used a clinical examination to sample selection. Inclusion criteria were: absence of mental or physical impairments (screened by a previous interview) and absence of the use of psychoactive or psychotropic substances. The subjects were instructed to abstain from smoking, taking alcohol, coffee, tea, cola, or any other drinks containing drugs, starting at least 24 h prior to the test day. Subjects with previous experience in typewriting were excluded from the experiment. All subjects signed a consent form and were aware of all the experimental protocol. The experiment was approved by the Ethics Committee of Federal University of Rio de Janeiro (IPUB/UFRJ).

The subjects were randomly distributed in three groups: placebo ($n = 13$), bromazepam 3 mg ($n = 13$) or bromazepam 6 mg ($n = 13$). The first EEG acquisition was performed, in a resting, pre-task condition. Then, the subjects were exposed to placebo or bromazepam 3 mg or bromazepam 6 mg, respecting a randomized double-blind design. Forty minutes after drug ingestion, the typewriting task started concomitantly with EEG recording. Finally, a last EEG acquisition was performed in a rest condition (i.e. post-task).

To execute 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 position of the hands. 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, the space key was pressed using the left or right thumb. Individuals were required to employ typewriting movements with maximum velocity and accuracy. The experimental task demanded sustained attention and it was performed in a predictable scenario (i.e., skill closed). The motor behavior's results were also published elsewhere [6].

The International 10/20 System for electrodes 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 monopole 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 were kept under 5–10 kΩ. Visual inspection and independent component analysis (ICA) were applied to remove possible sources of artifacts produced by the task. 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 *DataAcquisition* (Delphi 5.0), developed at the Brain Mapping and Sensorimotor Integration Laboratory was employed to filter the raw data: *notch* (60 Hz), high-pass of 0.3 Hz and low-pass of 25 Hz. Quantitative EEG parameters were extracted from six different moments: before, during (i.e. blocks 1, 2, 3 and 4) and after the execution of the task. The analyzed neurophysiologic parameter was the relative power in *theta*, *alpha* and *beta* bands. To obtain reference-free data, 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.). In order to compute the EEG average, 140 (±50S.D.) epochs were extracted from the raw data for each block. The relative power value (i.e. a percentage of the total power) was estimated for each epoch based on numerical integration of PSD in each considered EEG band, resulting in a set of values for each moment. These sets of relative power estimates were then submitted to statistical testing.

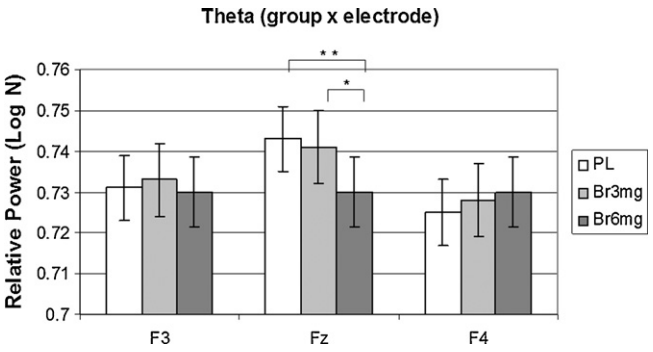

Fig. 1. Mean and standard deviation for relative power on theta band. An interaction was observed between group and electrode factors. (*) Significant difference, $P < 0.05$. (**) Significant difference, $P < 0.001$.

To analyze the EEG variables, a three-way ANOVA (repeated measures) was performed for each frequency bands: theta (4–6 Hz), alpha (7–12 Hz) and beta (14–25 Hz). Three main factors were combined: group (i.e., PL × Br 3 mg × Br 6 mg), blocks (i.e., block 1 × block 2 × block 3 × block 4) and electrodes (i.e., F3, Fz, F4, C3, Cz, C4, P3, Pz, P4). A constant (i.e., 2) was added to the EEG relative power. The values were log-transformed by SPSS software (version 14.0) to approximate a normal distribution. Scheffe post hoc was applied ($p < 0.05$).

An interaction between group and electrodes was observed ($F_{16,23} = 0.75$; $P < 0.05$). Post hoc analyses indicated a power reduction in the Br 6 mg when compared with PL ($t_{12} = 3.14$; $P < 0.01$) and with Br 3 mg ($t_{12} = 2.58$; $P < 0.05$) only in the electrode Fz (see Fig. 1). No differences were seen between Br 3 mg and PL. No other significant result was observed in the theta band.

A main effect for electrode was observed ($F_{8,31} = 24.5$, $P < 0.001$). Post hoc analyses indicated a power decreasing in medial–frontal electrodes (i.e., F3 and F4) when compared with all others ($P < 0.001$). On the other hand, the highest power values were observed in parietal electrodes (i.e., P3, Pz and P4) ($P < 0.001$). The power values registered in electrodes in central gyrus (i.e., C3 and C4) were higher when compared with F3 and F4 ($P < 0.001$) and lower when compared with electrodes P3 and P4. No other significant result was observed in the alpha band.

An interaction between group and electrodes was observed ($F_{16,23} = 2.86$; $P < 0.05$). Post hoc analyses indicated a higher power level in the PL when compared with Br 3 mg ($t_{12} = 2.51$; $P < 0.05$) and with Br 6 mg ($t_{12} = 2.05$; $P < 0.05$) only in the electrode F3. No differences were observed between Br 3 mg and Br 6 mg (Fig. 2). No other significant result was observed in the beta band.

The aim of this study was to investigate the influence of bromazepan on the EEG and the motor learning process when subjects were submitted to a typewriting task. We investigated bromazepam due to its abuse on different populations and its high prevalence among older individuals which are more sensitive to

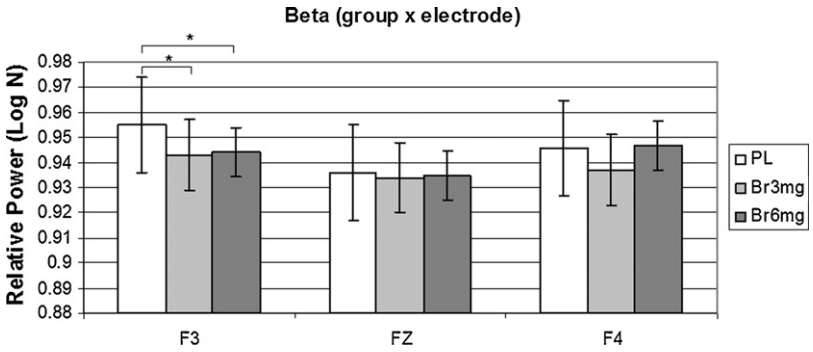

Fig. 2. Mean and standard deviation for relative power on beta band. An interaction was observed between group and electrode factors. (*) Significant difference, $P < 0.05$.

the negative effects of a long half-life benzodiazepine [2]. A randomized double-blind design was applied to subjects distributed in three groups: placebo, bromazepam 3 mg and bromazepam 6 mg.

Our findings indicated that subjects who took Br 6 mg presented lower theta power values, particularly in the electrode Fz, when compared with PL and Br 3 mg. Smith and colleagues [20] reported an increasing in theta power, recorded in Fz, associated with general fatigue or decreasing in alertness from the start to end of a visual–motor task training session. According to this electrophysiological hypothesis, our data showed that 6 mg of Br can either decrease fatigue and/or enhance alertness of the subjects into a narrowed perceptive field. However, in Smith's experiment a task which involved simultaneous attention to multiple subtasks (i.e., space fortress game) was employed. On the other hand, our task involved a "strict perceptive field of view" without the necessity for subjects' engagement in multiple subroutines. Previous findings had already demonstrated modifications in the theta band in cortical frontal regions during several sensorimotor tasks [3,4,20]. In a study combining EEG with magnetoencephalography (MEG), two sources were estimated to analyze theta activity in prefrontal–medial superficial cortex and anterior cingulate cortex (ACC) during an experimental task demanding sustained attention [3]. The literature mentions the importance of such regions for planning and focal attention processes [3,19,20]. The Br 6 mg could contribute to a reduction in load of information to the frontal cortex. It justifies the lower power values observed in the electrode Fz. This reduction in load can be understood in two manners: increase in the accuracy due to reduction of peripheral information interference or narrowing the subjects' perception field by a decreasing in sensory information distribution. The significance of this mechanism for motor performance appears to depend on the nature of the task. The measurement of the brain electrical reactivity (e.g., event-related-potential or ERPs) suggests that the effect of bromazepam is already manifest in the early stages of information processing (attention–detection) as mirrored by drug effect on N1 amplitude [13]. The reduction of N1 amplitude in Leeuwen's experiment might point to reduced stimulus detection ability. Subjects under influence of bromazepam probably deal with less clear or evident signals for their final evaluation. Therefore, bromazepam deteriorates the ability of detecting relevant information in the close environment.

Our findings also indicated a main effect of electrode relative to the alpha band, but that the alpha activity was not significantly affected by bromazepam. Thus, the topographic distribution of alpha was similarly maintained for all groups. Alpha power is inversely related to the proportion of cortical (motor) activation, as indicated in other studies [1,22]. Our findings suggest increased cortical activity during the typewriting task, in the electrodes F3 (left hemisphere) and F4 (right hemisphere), placed over the premotor cortex. According to other experiments, the premotor cortex is activated during the motor planning phase of the contralateral limb movements [22]. Our experiment involves a bimanual task, thus, both left and right pre motor cortices. Additionally, a lower power level in electrode F4 was observed when compared with the electrode F3. Therefore, the subjects presented a higher activation in the right pre motor cortex related to motor planning of the left limb [12]. It may be associated with a higher effort to control the sequential finger movements in the non-dominant hand.

Our results indicated that subjects who took Br 3 mg or Br 6 mg presented lower power values in the beta band when compared with PL, particularly in the electrode F3. Experimental data have suggested a relationship between beta rhythms and sensorimotor demands. Subjects who were exposed to sensorimotor tasks presented beta reactivity in different regions of the somatosensory cortex, including both pre- and post-central gyrus [1,22]. Along this line, different experiments have showed a decreasing in power (e.g., ERD) when subjects were exposed to motor tasks involving the upper limb (e.g., hand or finger). Such reduction in power may suggest an increasing in magnitude of activation in specific cortical areas requested for the sensorimotor action [21,22]. Our findings suggest that Br 3 mg and Br 6 mg groups increases specific cortical sensorimotor components activation during the motor learning task. Our interpretation is that the decreasing in power induced by the motor task indicates a participation of the premotor cortex in acquisition and consolidation of procedural memory related to the sensorimotor performance [18]. According to another study, the brain uses a neurophysiologic mechanism that increases the focal activation of specific cortical areas and decreases the activity in other peripheral regions that are not relevant to the motor task [15]. In our experiment, the focal activity in the electrode F3, observed in Br 3 mg and Br 6 mg groups, suggest an intensified effect of the drug in the focal activation/surround deactivation mechanism. In this sense, the bromazepam can promote a perceptive narrowness involving the subjects to process only obvious characteristics of the task execution, and in parallel, disregard other environmental stimulus. This notion corroborates to justify the higher focal activation over the electrode F3 and lower activity over surround regions particularly in Br 3 mg and Br 6 mg groups.

Our results, regarding three EEG rhythms, suggest an important effect of the Br over the CNS during the motor learning process. According to the present analysis, two cerebral bands were influenced by the effect of Br. Specifically, theta (i.e., attention demands) and beta (i.e., somesthetic processing and spatial coordinates of the upper limb). Thus, the Br appears to change the functional coupling between the planning of the task which is related to the attention mechanisms (i.e., frontal cortex) and sensorimotor integration. Therefore, possible changes in motor performance caused by Br can be due to the influence of sensory variables, which are important to planning or motor programming of voluntary movements.

Such pattern of results suggests a dual influence: bromazepam aids motor performance during tasks that involve a predictable environment, as the typewriting task [6]; on the other hand, it may decrease motor performance in tasks executed in unstable scenarios as in the reaction time paradigms [10,13]. Tasks involving decision-making and immediate response to unanticipated stimuli may be more impaired by bromazepam and, thus, remains to be systematically studied. The present study presents as limitation the use of normal individuals as experimental subjects.

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References

[1] M. Alegre, A. Labarga, I. Gurtubay, J. Iriarte, A. Malanda, Beta electroencephalograph changes during passive movements: sensory afferences contribute to beta event-related desynchronization in human, Neurosci. Lett. 331 (2002) 29–32.
[2] J. Alvarenga, A. Filho, J. Firmo, M. Costa, E. Uchoa, Prevalence and sociodemographic characteristics associated with benzodiazepines use among community dwelling older adults: the Bambuí health and aging study, Rev. Bras. Psiquiatr. 30 (2007) 7–11.
[3] H. Asada, Y. Fukuda, S. Tsunoda, M. Yamaguchi, M. Tonoike, Frontal midline theta rhythms reflect alternative activation of prefrontal cortex and anterior cingulate cortex in humans, Neurosci. Lett. 274 (1999) 29–32.
[4] M. Bastiansen, J. Berkum, P. Hagoort, Event-related theta power increases in the human EEG during online sentences processing, Neurosci. Lett. 323 (2002) 13–16.
[5] M. Bourin, J. Auget, M. Colombel, C. Larousse, Effects of single oral doses of bromazepam, buspirone and clobazam on performance tasks and memory, Pharmacopsychiatry 22 (1989) 141–145.
[6] M. Cunha, D. Machado, V. Bastos, C. Ferreira, L. Basile, M. Cagy, R. Piedade, P. Ribeiro, Neuromodulatory effect of bromazepam on motor learning: an electroencephalographic approach, Neurosci. Lett. 407 (2006)166–170.
[7] M. Grunwald, T. Weiss, W. Krause, L. Beyer, R. Rost, I. Gutberlet, H. Gertz, Theta power in the EEG of humans during ongoing processing in a haptic object recognition task, Cogn. Brain Res. 11 (2001) 33–37.
[8] V. Hobi, U. Dubach, M. Skreta, I. Forgo, H. Riggenbach, The effect of bromazepam on psychomotor activity and subjective mood, J. Int. Med. Res. 9 (1981) 89–96.
[9] V. Hobi, U. Dubach, M. Skreta, I. Forgo, H. Riggenbach, The subacute effect of bromazepam on psychomotor activity and subjective mood, J. Int. Med. Res. 10 (1982) 140–146.
[10] A. Jansen, M. Verbaten, J. Slangen, Acute effects of bromazepam on signal detection performance, digit symbol substitution test and smooth pursuit eye movements, Neuropsychobiology 20 (1988) 91–95.
[11] I. Jenkins, D. Brooks, P. Nixon, R. Frackowiak, R. Passingham, Motor sequence learning: a study with positron emission tomography, J. Neurosci. 14 (1994) 3775–3790.
[12] E. Kandel, S. Schwartz, T. Jessel, Principles of Neuroscience, McGraw-Hill, New York, 2000.
[13] V. Leeuwen, M. Verbaten, H. Koelega, J. Kenemans, J. Slangen, Effects of bromazepam on single-trial event-related potentials in a visual vigilante task, Psychopharmacology 106 (1992) 555–564.
[14] M. Llorente, D. David, A. Galden, M. Silverman, Defining patterns of benzodiazepines use in older adults, J. Geriatr. Psychiatry Neurol. 13 (2000) 150–160.
[15] C. Neuper, G. Pfurtscheller, Event-related dynamics of cortical rhythms: frequency-specific features and functional correlates, Int. J. Psychophysiol. 43 (2001) 41–58.
[16] E. Niedermeyer, F. Lopes da Silva, Electroencephalography: Basic Principles, Clinical Applications and Related Fields, Urban & Schwarzenberg, Baltimore–Munich, 1999.
[17] R. Oldfield, The assessment and analysis of handedness: the Edinburgh inventory, Neuropsycology 9 (1971) 97–113.
[18] T. Schack, F. Mechsner, Representation of motor skills in human long-term memory, Neurosci. Lett. 391 (2006) 77–81.
[19] S. Slobounov, K. Fukada, R. Simon, M. Rearick, W. Ray, Neurophysiological and behavioral indices of time pressure effects on visuomotor task performance, Cogn. Brain Res. 9 (2000) 287–298.
[20] M. Smith, L. McEvoy, A. Gevins, Neurophysiological indices of strategy development and skill acquisition, Cogn. Brain Res. 7 (1999) 389–404.
[21] A. Stancák, G. Pfurtscheller, Desynchronization and recovery of beta rhythms during brisk and slow self paced finger movements in man, Neurosci. Lett. 196 (1995) 21–24.
[22] W. Szurhaj, P. Derambure, E. Labyt, F. Cassim, J. Bourriez, J. Isnard, J. Guieu, F. Mauguière, Basic mechanisms of central rhythms reactivity to preparation and execution of a voluntary movement: a stereoelectroencephalographic study, Clin. Neurophysiol. 114 (2003) 107–119.