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Gamma band oscillations under influence of bromazepam during a sensorimotor integration task: An EEG coherence study

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ABSTRACT

The goal of the present study was to explore the dynamics of the gamma band using the coherence of the quantitative electroencephalography (qEEG) in a sensorimotor integration task and the influence of the neuromodulator bromazepam on the band behavior. Our hypothesis is that the needs of the typewriting task will demand the coupling of different brain areas, and that the gamma band will promote the binding of information. It is also expected that the neuromodulator will modify this coupling. The sample was composed of 39 healthy subjects. We used a randomized double-blind design and divided subjects into three groups: placebo ($n = 13$), bromazepam 3 mg ($n = 13$) and bromazepam 6 mg ($n = 13$). The two-way ANOVA analysis demonstrated a main effect for the factors condition (i.e., C4–CZ electrode pair) and moment (i.e., C3–CZ, C3–C4 and C4–CZ pairs of electrodes). We propose that the gamma band plays an important role in the binding among several brain areas in complex motor tasks and that each hemisphere is influenced in a different manner by the neuromodulator.

During motor learning, it is possible to note different phases of learning in the execution of a motor act. The motor control migrates from a stage that is initially explicit to a stage that is almost automatic. In terms of behavior, this automation is characterized by fast and coordinated movements as well as a lower occurrence of errors [11]. Cortically, to obtain this quality of control, sensorimotor integration is fundamental. It is the continuous processing, by the motor system, of sensory afferents which prepares for motor acts and enhances the execution of fine motor activities. In this process, the central nervous system (CNS) integrates information coming from multiple sensory channels, allowing the performance of specific, goal-directed tasks [20,21]. The cerebral cortex is composed of cortical areas that are neither purely sensory nor purely motor, but associative, and serve higher-order integrative functions. These sites of the cortex called association areas, associate sensory inputs with motor response and perform those mental processes that intervene between sensory inputs and motor outputs [24].

Sensorimotor integration behaves in a cooperative manner, as do other cognitive functions. It depends on the exchange of essential information arising from different brain sites [39,40]. Communication among brain structures is thought to be implemented by coupling or synchronization. This process allows neural assemblies, which are spatially distant, to work simultaneously [31]. One method to measure the coupling is to use the coherence function of the quantitative electroencephalography (qEEG) [37]. This function enables us to quantify how much two brain sites are functioning with similarity. The EEG can record the sum of the activity of millions of neurons through their dendritic activity. Different from other modern techniques of neuroimaging, the EEG measures the neural activity in real time, making it a useful tool to study the interactions between brain areas and neural networks dynamics [30].

The gamma frequency band (between 30 and 100 Hz) of the EEG has been explored in animal and human models to understand its role in the cortical oscillatory dynamics [17,16,36]. Gamma-band oscillations are thought to provide a mechanism for the binding of functionally related cortical elements such as attention,

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memory, motor planning, sensorimotor integration and cognition [10,15,19,23,25,28]. Recent data demonstrated through synaptic models that gamma plays an important role in the processes of memory and induction of time-dependent neuronal spiking. It is also suggested that the GABA A receptor works for the CNS as a modulator and synchronizer of neural networks [14]. This peculiarity of the GABA A receptor makes the use of a neuromodulator, such as bromazepam, interesting, because it increases the affinity of the receptor for its neurotransmitter [26]. This increase in affinity intensifies the action of the neurotransmitter, modifying neural activity and consequently may influence the neural networks behavior [3].

In this context, the goal of the present study was to explore the dynamics of the gamma band using the coherence function of the qEEG in a sensorimotor integration task and observe the influences of the neuromodulator bromazepam on the band behavior. Even though the experimental task utilized in this study has been employed in other studies [3,5,6], no previous experiment has analyzed gamma. This study, therefore, fills a research gap by providing new evidence about gamma behavior during this type of task. Our hypothesis is that the needs of the task (i.e., attention, spatial memory, planning and execution of complex finger movements) will demand the coupling of different brain areas, and that the gamma band will promote the binding of information. We also expect that the neuromodulator will modify this coupling.

Sample was composed of 39 healthy subjects (15 male and 24 female; aging between 20 and 40 years old), right-handed according to the Edinburgh inventory [27]. Inclusion criteria were: absence of mental or physical impairments and absence of the use of psychoactive or psychotropic substances (screened by a previous anamnesis and clinical examination). 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 experiment day. Subjects with previous experience in typewriting were excluded from the experiment. All participants 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 3 groups: placebo ($n = 13$), bromazepam 3 mg ($n = 13$) or bromazepam 6 mg ($n = 13$). Therefore, the experiment follows a randomized double-blind design. The first EEG acquisition was performed before the ingestion of placebo, 3 or 6 mg of bromazepam. One hour after the ingestion of placebo or bromazepam, the typewriting task was performed concomitantly with EEG recording. Immediately and 30 min after motor task execution, new data were recording.

The task was performed in a sound and light-attenuated room, to minimize sensory interference. Individuals sat on a comfortable chair to minimize muscular artifacts, while electroencephalography (EEG) was done. To execute the task, 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 task was composed of three 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. Individuals were required to employ typewriting movements with maximum velocity and accuracy. The letter chosen for data analysis was the letter h (i.e., relating to right hand). The motor behavior results were also published elsewhere [3,5].

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 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. 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.

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. Data from individual electrodes exhibiting loss of contact with the scalp or high impedances (>10 kΩ) were deleted and data from single-trial epochs exhibiting excessive movement artifact (±100 μV) were also deleted. Independent component analysis (ICA) was then applied to identify and remove any remaining artifacts after the initial visual inspection. ICA is an information maximization algorithm that derives spatial filters by blind source separation of the EEG signals into temporally independent and spatially fixed components. Independent components resembling eye-blink or muscle artifact were removed and the remaining components were then back-projected onto the scalp electrodes by multiplying the input data by the inverse matrix of the spatial filter coefficients derived from ICA using established procedures. The ICA-filtered data were then reinspected for residual artifacts using the same rejection criteria described above. Then, 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 following moments: before, during, immediately after and 30 min after motor task execution. The analyzed electrophysiological variable was gamma (35–60 Hz) coherence. It represents a measurement of linear covariation between two signals in the frequency domain. It is mathematically bounded between zero and one, whereby one signifies a perfect linear association and zero denotes that the signals are not linearly related at that particular frequency. The premise is that when activities from spatially remote events covary they tend to interact, also denoted as functional connectivity. Standard coherence as a measure of functional coupling provides a link between two signals but no directional information. To this end, estimators can be constructed, such as a directed transfer function, which examines asymmetries in inter-regional information flow and establishes a direction of drive between the coupled sites [18,33,35].

In the statistical analysis, an ANOVA two-way and a Scheffé post hoc test were applied to analyze the combinations of pairs of electrodes of interest, i.e., C3–CZ, C3–C4 and C4–CZ ($p \leq 0.05$). In this sense, the following factors were analyzed: moment (before × task × after × 30 min after) and condition (Placebo × Br 3 mg × Br 6 mg).

The C3 and C4 electrodes represent the primary sensory motor cortex (SM1) in each hemisphere that is functionally linked to motor preparation and execution [37]. The CZ electrode represents the SM1 of both hemispheres and the supplementary motor area (SMA), which is functionally related to temporal organization and coordination of sequential movements [38]. The gamma band was chosen to explore its associations with the binding for sensorimotor integration, attention and motor planning [28]. The C3–C4 electrode analysis demonstrates main effect for moment ($p < 0.001$) and the *Scheffé post hoc* analysis identified a significant difference among the moment *task* with the other moments: moment

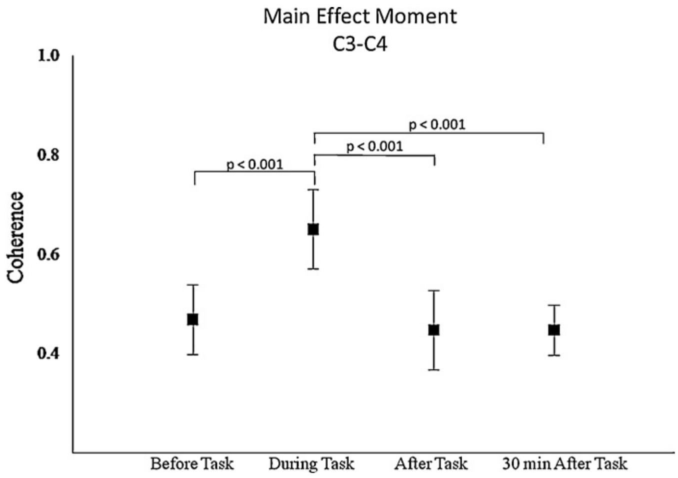

Fig. 1. Mean and standard deviation for coherence on gamma band. Main effect for moment observed in the C3–C4 electrode pair.

before ($p < 0.001$), moment *after* ($p < 0.001$) and moment 30 min *after* ($p < 0.001$) (Fig. 1) The C3–CZ electrode analysis demonstrates main effect for moment ($p < 0.001$) and the *Scheffé post hoc* analysis identified a significant difference among the moment *task* with the other moments: moment *before* ($p = 0.016$), moment *after* ($p = 0.001$) and moment 30 min *after* ($p < 0.001$) (Fig. 2). The C4–CZ electrode analysis demonstrates main effect for moment ($p = 0.003$) and the *Scheffé post hoc* analysis identified a significant difference among the moment *task* with the other moments: moment *before* ($p = 0.036$), moment *after* ($p = 0.039$) and moment 30 min *after* ($p = 0.011$). This electrode pair analysis also showed main effect for condition ($p < 0.001$) and the *Scheffé post hoc* analysis identified a significant difference among the condition *brom* 6 mg with the other conditions: condition *placebo* ($p = 0.001$) and condition *brom* 3 mg ($p = 0.016$) (Fig. 3).

The goal of the present study was to explore the dynamics of the gamma band using the coherence function of the qEEG in a sensorimotor integration task and observe the influences of the neuromodulator bromazepam on the band behavior. Even though the experimental task utilized in this study has been employed in other studies [3,5,6], no previous experiment has analyzed gamma. This study, therefore, fills a research gap by providing new evidence about gamma behavior during this type of task. Our hypothesis is

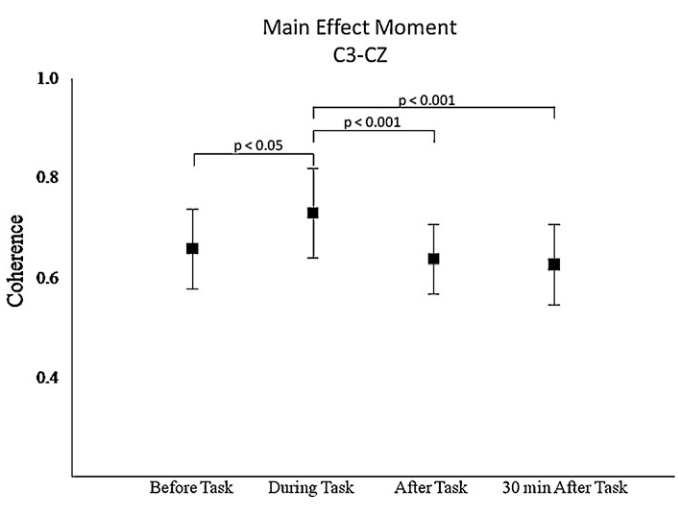

Fig. 2. Mean and standard deviation for coherence on gamma band. Main effect for moment observed in the C3–CZ electrode pair.

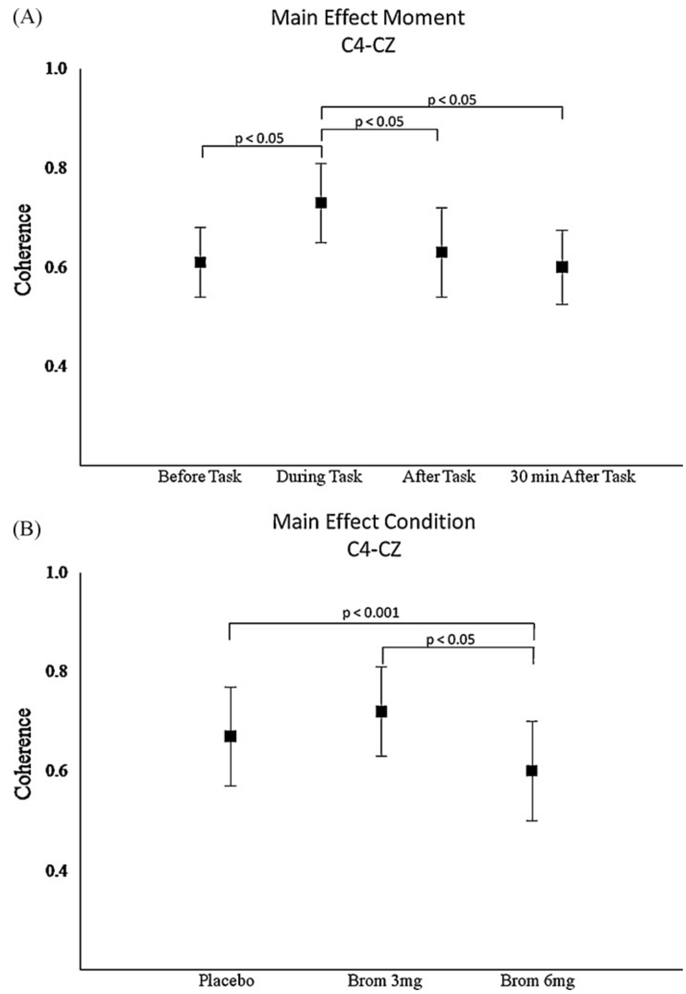

Fig. 3. (A) Mean and standard deviation for coherence on gamma band. Main effect for moment observed in the C4–CZ electrode pair. (B) Mean and standard deviation for coherence on gamma band. Main effect for condition observed in the C4–CZ electrode pair.

that the needs of the task (i.e., attention, spatial memory, planning and execution of complex finger movements) will demand the coupling of different brain areas, and that the gamma band will promote the binding of information. We also expect that the neuromodulator will modify this coupling.

The discussion is divided into two parts. First, we discuss the main effect for condition (e.g., C4–CZ electrodes pair) to understand how the neuromodulator modified the coupling relationship. Next, we focus on the discussion of the result of main effect for moment observed in all electrode pairs of interest and their relation with the gamma band. Finally, we present the importance, concluding remarks and limitations of the study.

In terms of the neuromodulator's effect on the coupling, we found differences in the coherence values for the C4–CZ electrode pair. At this site, the *brom* 6 mg condition showed the lowest coherence values compared with the *placebo* condition and the *brom* 3 mg condition. The hemispheric specialization [34] could be a reasonable explanation for our data. This hypothesis suggests that each hemisphere has specific functions and contribute to motor control in distinctive ways. The right hemisphere uses attention and sensory feedback to create spatial References On the other hand, the left hemisphere plays a dominant role on the planning and execution of the movement, also in bimanual tasks [35]. In this sense, the right hemisphere (C4–CZ), which is more involved with sensorial needs, could have been more strongly affected by the drug.

Inversely, the left hemisphere (C3–CZ) and the interhemispheric communication (C3–C4), both highly requested [2,8,32,33] by the task was not affected by the drug. Several studies that combined the usage of bromazepam in motor tasks demonstrated varied results [3–7,12]. Some of them suggested that the bromazepam could aid the cognitive and motor performance [3,5–7]. However, other studies argued that this drug could impair psychomotor capacity [4,12]. The present study differs from other papers by analyzing new variables: gamma and coherence. Since the literature lacks data regarding the use of this neuromodulator with these variables, we could only speculate about the results of our experiment.

Relative to the results of main effect for moment, we observed an increase of coherence values during the task performance. This behavior was verified in all electrode pairs analyzed (e.g., C3–CZ, C3–C4 and C4–CZ). This moment of the task involved more complexity because the subject needed to execute several cognitive functions. Cortically, we assume that this enhance of the coherence values reflects the necessity of the cooperation of several brain structures to perform this cognitive-motor function. The SM1 receives somatosensory information from speed, trajectory and spatial position of the limb, which are important in the movement's execution. In addition, the SMA plays an essential role in the temporal organization and coordination of sequential movements [34]. It is suggested that gamma promoted the binding of information through the coupling of these sites, to supply task demands. According to Pfurtscheller and Lopez da Silva [29], beta and alpha frequency bands are too slow to be used as signal carriers for the binding in high levels of processing. In contrast, gamma band is considered to be ideal for establishing rapid synchronization between neural sites. The relationship between a higher level of complexity and an elevation of coherence values receives support from findings in other studies that use the coherence function in manual tasks [1,9,22]. In an experiment that used a task with four different finger movements and escalating complexity levels, Manganotti et al. [22] verified higher values of task-related coherence (TRCoh) in alpha and beta bands (8–12 and 13–20 Hz, respectively) during the more complex movements. In another experiment, Gerloff et al. [9] explored the coupling among several motor areas (premotor cortex, SM1 and SMA) during simple, internally and externally paced finger movements. They observed changes in the TRCoh values of the beta band (20–22 Hz) and proposed that greater functional coupling of cortical premotor and sensorimotor areas is associated with the internal pacing of movement that was considered more complex by the authors. Andres and Gerloff [1] suggested that higher information flow between brain sites is necessary to implement difficult movements. This increase in TRCoh values may reflect this elevated information flow. If these works had also explored the gamma band, they would have found similar results to our data. In summary, as the first investigation, to our knowledge, exploring the gamma band behavior in a typewriting task and how the usage of a neuromodulator can modify its behavior, the present study contributes to the existing literature. We proposed that gamma plays an important role in promoting binding of several brain areas in complex motor tasks and that each hemisphere was influenced in a different manner by the neuromodulator. Future experiments that utilize the same variables with new populations and methods are necessary to expand the knowledge about gamma behavior and the effects of neuromodulators. The present study was limited to healthy individuals as experimental subjects.

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