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ORIGINAL RESEARCH

Gamma absolute power reveals activation of motor areas after hand immobilization

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OBJECTIVE: To analyze changes in gamma band absolute power in motor cortical areas, before and after a condition of hand immobilization for 48 hours.

METHOD: Fifteen healthy volunteers, aged between 20 and 30, were submitted to EEG assessment before and after 48 hours of immobilization of the dominant hand, while performing a motor task triggered by a visual stimulus. A two-way repeated measures ANOVA with two within-group factors (moment x condition), each one with two levels (before *vs.* after visual stimuli; before vs. after 48-hour HI, respectively) was used to test for changes in beta band absolute power.

RESULTS: Statistical analysis revealed that hand immobilization caused changes in cortical areas. A significant increase in gamma band absolute power was found after hand immobilization at electrodes F3 ($p = 0.001$) at F4 ($p = 0.001$) and at Fz ($p = 0.001$), at C3 ($p = 0.001$), C4 ($p = 0.001$) and Cz ($p = 0.001$).

CONCLUSION: These results reveal that oscillations of the gamma band can be a cortical strategy to solve the effect of less activation due to movement restriction. Knowledge of the functioning of motor cortical areas after a condition of immobilization can lead to more effective strategies in rehabilitation.

KEYWORDS: Gamma band, EEG, Hand, Immobilization, Neural plasticity, Electroencephalography.

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INTRODUCTION

It is well established that immobilization results in changes of skeletal muscle properties. Reports are to be found about cast immobilization and other forms of disuse, identifying factors that can result in atrophy, increased intramuscular connective tissue, reduction of muscular strength and impairment to motor function.[1,2] These effects have been shown to be preceded by neural adaptations.[3]

Other studies involving nerve stimulation, transcranial magnetic stimulation, magnetic resonance imaging, electromyography (EMG) and electroencephalography (EEG) reported that changes of cerebral functions can cause impairment in the activation of skeletal muscle by the nervous system.[3-5] Thus, studies about implications of immobilization on neuroplasticity may be useful for the development of strategies in therapeutic interventions, guiding best practice in rehabilitation.[3]

The EEG measures the spontaneous cortical electrical activity and can be helpful in terms of identifying, monitoring and classifying bioelectrical signals into frequency bands, and correlating such signals to wakefulness/not wakefulness. Moreover, EEG registers may be potentially useful in the measurement of athletic training and rehabilitation processes.[6] Specific

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frequency bands are related to motor behavior, to levels of consciousness and to essential conditions for learning.[7] Particularly, the Gamma band (in the frequencies of 30-100Hz) is associated to sensory and motor processes required for motor control. This band is responsible for keeping the selective attention processes necessary for motor learning;[8] there is evidence that the gamma bands correlate well to changes of blood oxygen level-dependent (BOLD) contrast imaging in functional Magnetic Resonance Imaging (fMRI) during cognitive processing. Furthermore, the gamma band seems to be linked to enhanced neural communication, reflecting cortical arousal.[9,10]

The gamma band plays an important role in the binding between several brain areas in complex motor tasks.[11] We may suppose that after a condition of immobilization this EEG frequency would be more pronounced, as a means of supporting communication of neural populations in cortical regions that had been less activated due a condition of immobilization. For instance, a study using MRI[12] examined subjects with injury of the right upper extremity that were immobilized for at least 14 days. This experiment showed decreased cortical thickness in the sensorimotor regions (left primary motor and somatosensory area) and a decrease in the fractional anisotropy of the left corticospinal tract after immobilization. This finding reflected plastic changes in gray and white matter, leading to the understanding that immobilization induces rapid reorganization of the sensorimotor system. Although there is a cortical involvement in motor restriction, the role of gamma band absolute power in this situation is not well understood; this is especially true within the time period which occurs in the few hours after immobilization.

Our hypothesis was that an increase of gamma absolute power occurs after hand immobilization to allow communication between cortical regions and elicit movement. The absence of movement can affect the activation of sensory motor integration areas and the functions of these areas may perhaps be replaced by the activation of other regions. The EEG may be useful to understand how immobilization changes the activity of cortical areas induced by motor task performance. Thus, the goal of this study was to analyze changes of gamma band absolute power in motor cortical areas (frontal and central regions) before and after a condition of immobilization of the dominant hand for a period of 48 hours. An associated article on beta absolute power in the same procedural setup is published simultaneously with this report.[13]

METHODS

Sample

Fifteen right-handed healthy subjects, 4 men and 11 women (average age: 24 ± 1.2 years) gave their written informed consent to participate in the experiment. They were chosen randomly and the recruitment of the volunteers was accomplished through a research announcement posted in different Universities in the State of Rio de Janeiro. Exclusion criteria were mental or physical illnesses and the use of psychoactive or psychotropic substances during the entire period of the study. Due to hand laterality, the Edinburgh inventory[14] was used to identify the laterality predominance of the participants (right-handed *vs* left-handed). Left-handed individuals were also excluded from the experiment. The subjects were instructed not to use tobacco, coffee[15] or alcoholic drinks[16] 10 hours before the test because these substances may influence cortical activation recorded by QEEG brain mapping.[17,18] The study was approved by the ethics committee of Veiga de Almeida University and complied with the ethical standards of the Declaration of Helsinki.

Tasks and Procedures

A room with acoustic and electrical isolation was used so that the experimental procedure could be carried out. The lights were dimmed during the electroencephalography (EEG) signal acquisition period; subjects were sitting in a chair with armrest in order to minimize muscle artifact during EEG signal acquisition. In front of the subjects, on a table, there was a 15-inch monitor that was placed facing the subjects and turned on only when the subjects performed the task (i.e., flexion and extension of the index finger). Initially, the EEG signal acquisition lasted for 2 minutes (at rest) with the monitor turned off and facing the subjects. Then, an accelerometer was placed on the right index finger. A visual stimulus appeared on the monitor and the subjects performed the task (i.e., flexion and extension of the index finger). The accelerometer was connected to the EEG though an additional channel (i.e., channel 21) and provided a signal to relate the EEG recording to the movement.

The task required the participants to flex and extend their index finger when visual stimuli were generated as random images (namely a yellow ball) on the monitor. The complete task involved 6 blocks of 15 trials. In order to avoid muscle fatigue, a 3-minute break between each block was given to the subjects. Thus, the task lasted 1 minute for each block with 3-minute intervals between blocks, adding up to 24 minutes for the entire task. After completion of the task, the monitor was turned off and the subjects were submitted again to EEG during 2 minutes, at rest. After this EEG recording, a plaster cast was applied to the subjects' right hand and kept on for 48 hours; the cast was applied in the hand and fingers in the flexed position in order to prevent any hand or finger movement. After 48 hours, subjects returned to the laboratory to remove the plaster cast. Five minutes after removal, they were submitted to the same task procedures that had been performed before immobilization.

Data acquisition - Electroencephalography

The International 10/20 system for electrodes was used with 20-channel Braintech-3000 EEG system (EMSA-

Medical Instruments, Brazil). The 20 electrodes were arranged in a nylon cap (ElectroCap Inc., Fairfax, VA, USA), yielding mono-pole derivations to linked earlobes. Different sizes of the nylon cap were used according to the subject's cranial perimeter. In addition to those, two 9-mm-diameter electrodes were attached above and on the external corner of the right eye, in a bipolar electrode montage, to monitor eye movement artifacts (EOG). Impedance of EEG and EOG electrodes was kept under 5-10 KΩ. Acquired data had total amplitudes of less than 100 μV. The EEG signal was amplified with a gain of 22.000 times, analogically filtered between 0.3 Hz (high-pass) and 100 Hz (low-pass), and sampled at 240 Hz. A Delphi 5.0 Data Acquisition software was employed to filter the raw data with a 60 Hz notch filter.

Data processing

A visual inspection and independent component analysis (ICA) was applied to identify and remove any remaining artifacts, i.e., eye blinks and ocular movements.[17,18] ICA was applied to the EEG recordings in order to interpret the source of underlying electrocortical signals in the contaminated artifact of electrical potentials on the scalp. Data from individual electrodes exhibiting loss of contact with the scalp or high impedance levels (>10 kΩ) were discarded, and data from single-trial epochs exhibiting excessive movement artifacts (± 100 μV) were also deleted. ICA is an information maximization algorithm that blinds EEG signals related to the artifacts, it was applied to identify and remove any artifacts after the initial visual inspection.[17,18] Independent components resembling eye-blinks or muscle artifacts were removed and the remaining components were then projected back onto the electrode data by multiplying it by the inverse matrix of the spatial filter coefficients derived from ICA, using established procedures. The ICA-filtered data were then re-inspected for residual artifacts using the same rejection criteria described above. Then, a classic estimator was applied for the power spectral density, or directly from the square modulus of the Fourier Transform performed by MATLAB (Matworks, Inc.). Quantitative EEG parameters were reduced to 4 sec. periods (the selected epoch started 2 sec. before and ended 2 sec. after visual stimulus).

Selected derivations and frequency band

Because the experiment involved a motor task, derivations F3, F4 and Fz were selected due to frontal region relationships with motivation, planning and motor programming.[19] Derivations C3, Cz and C4, were also selected because of their relationship with the motor cortex.[20,21] The gamma band was chosen due to its relation with motor tasks and because it is the most specific EEG band to investigate movements patterns. It may reflect the efficiency of sensory-motor integration and indicates levels of coupling between cortical areas.[22,23]

Statistical Analysis

Data were normalized into values of absolute power using natural logarithms in order to approximate values to a normal distribution; normality and homoscedasticity were verified through the Levene and Shapiro-Wilk tests. A two-way repeated measures ANOVA with two within-group factors (moment x condition), each one with two levels (before *vs* after visual stimuli; before *vs* after 48-hour HI, respectively) was used to test for changes in gamma band absolute power. The significance levels were set at $p \leq 0.05$. The analyses were conducted using the SPSS for Windows version 18.0 (SPSS Inc., Chicago, Il, USA).

RESULTS

The statistical design allowed us to investigate motor cortical function before and after 48 hours of hand immobilization: more precisely, to determine whether gamma band absolute power is affected when a right handed subject performs index finger movements after 48 hours of immobilization of the dominant hand. The two-way ANOVA indicated a main effect for condition (i.e., before *vs* after immobilization), no effect for moment (before *vs* after task) and no interaction. We found an increase of the gamma band absolute power at F3, F4, Fz, C3, C4 and Cz derivations. In frontal regions (Figure 1), the two-way ANOVA found a main effect for condition at F3 [$F(1,3489)=106.074$; $p=0.001$; $\eta^2_p= 0.030$], at F4 [$F(1,3588)=170.233$; $p=0.001$; $\eta^2_p=0.045$] and at Fz derivations [$F(1,3927)=36.800$; $p=0.001$; $\eta^2_p= 0.009$]. Similarly, at central regions (Figure 2) that correspond to the motor cortex, the two-way ANOVA found an increased gamma absolute power after hand immobilization at C3 [$F(1,3705)=209.775$; $p=0.001$; $\eta^2_p= 0.054$], C4 [$F(1,3767)=30.696$; $p=0.001$; $\eta^2_p= 0.008$] and Cz [$F(1,3900)=60.954$; $p=0.001$; $\eta^2_p= 0.000$].

Particularly, at C3, the gamma band absolute power before hand immobilization presented a mean value of 0.024±0.01; after hand immobilization this value increased to 0.033±0.01. This was the greatest increase of any of the selected derivations: C3 corresponds to the cortical region responsible for motor control of the right hand. There were no significant moment x condition interactions nor were there any significant main effects for moment in any of the analyzed derivations.

DISCUSSION

We analyzed changes of the gamma band absolute power in motor cortical areas (frontal and central regions) before and after a condition of immobilization of the dominant hand for 48 hours.

The Gamma band is considered to be the most specific EEG signal to investigate movement patterns;[24] thus it could

Figure 1 - Mean and SE indicate main effect for condition (before immobilization versus after immobilization) observed in (A) left prefrontal cortex (F3) (p=0.001), (B) central prefrontal cortex (Fz) (p=0.001) and (C) right prefrontal cortex (F4) (p=0.001).

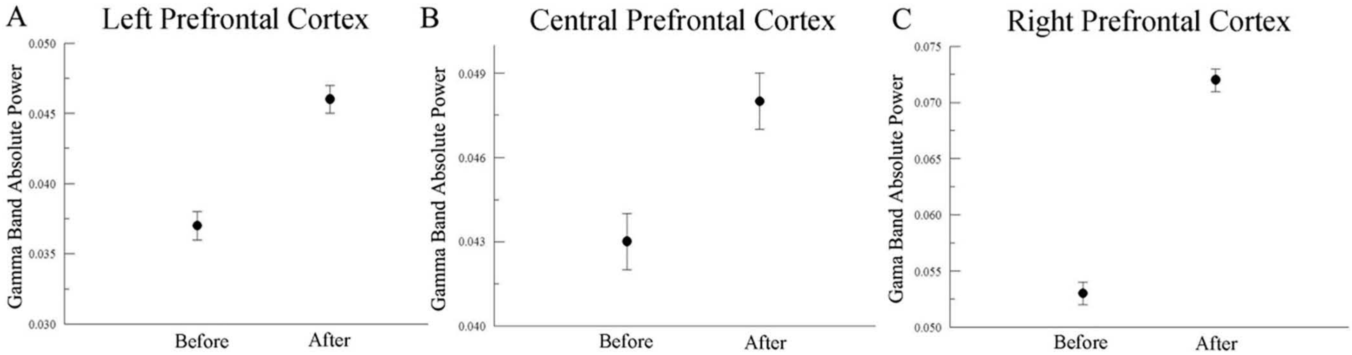
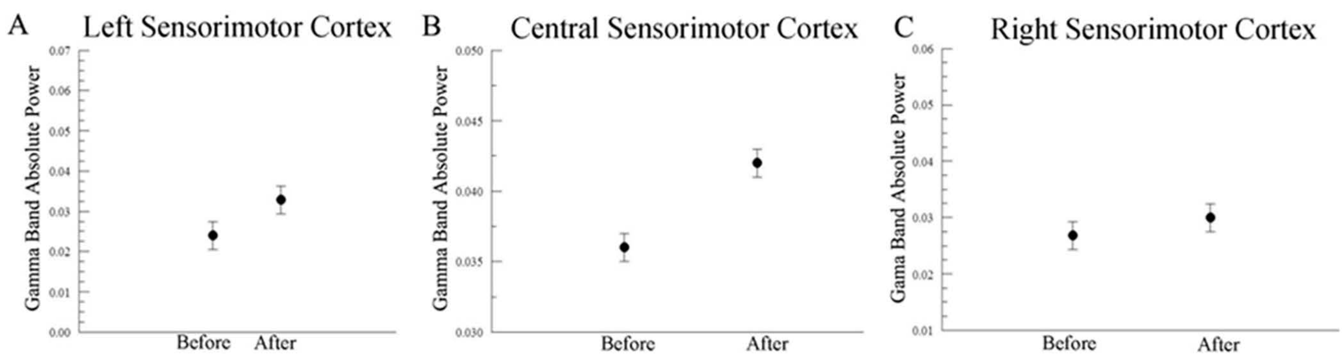


Figure 2 - Mean and SE indicate main effect for condition (before immobilization versus after immobilization) observed in (A) left sensorimotor cortex (C3) (p=0.001), (B) central sensorimotor cortex (Cz) (p=0.001) and (C) right sensorimotor cortex (C4) (p=0.001).



provide a better understanding about how motor cortical areas elicit movement after a condition of immobilization. Our hypothesis was that after immobilization, the gamma band frequency would be more pronounced in order to allow communication between neural populations of motor cortical areas that were less activated during the immobilization condition. The results showed a main effect of condition (i.e., before *vs* after immobilization) on gamma band absolute power. The analyzed derivations located in motor cortical areas (F3, F4, Fz, C3, C4 and Cz) exhibited an increase of gamma band absolute power when the volunteers executed the motor task after immobilization.

Our results point to an increase on gamma band absolute power on F3, F4, and Fz. Frontal cortical areas are associated motivation, planning, reasoning, problem solving and execution of voluntary movements.[25,26] Specifically F3 and F4 derivations (middle frontal gyrus) correspond to manual planning areas;[27,28] F3 is associated with the cortical area corresponding to right hand (the immobilized hand),while F4 associated with the left hand. Increased gamma band absolute power at F3 after hand immobilization indicates more activation at this cortical region in order to elicit the index finger movement required by the task. We also found an increase of gamma band absolute power at Fz and F4 derivation: at F4 the increase was greater than at F3 and Fz, indicating larger cortical arousal contralateral to the region corresponding to the immobilized right hand.

The contralateral activation of cerebral cortex after a neural lesion has been used as a therapeutic strategy to recover motor function in some movement disorders.[29,30] The immobilization of a healthy upper limb leads to an increased cortical activation at the contralateral region involved in the movement execution. This therapeutic approach, named constraint-induced movement therapy, is supported by the pattern of cortical activation due contralateral limb restriction.[31,32] This concept is reinforced by our findings regarding a larger increase of the gamma band absolute power at the F4 derivation; this shows that the homologous right side cortical region is activated to compensate for the left side region, which is responsible for the immobilized right hand. It thus appears that cortical reorganization did occur in spite of the fact that immobilization only lasted for 48 hours. The motor cortex can be seen here to elicit a compensatory functional strategy, an example of adaptive neuroplasticity.[4,33,34]

Sterling et al[29] reported results of functional magnetic resonance imaging (fMRI) in children with congenital hemiparesis submitted to constraint-induced movement therapy: their results showed increased gray matter volume in the sensorimotor cortex contralateral to the more-affected arm; they also showed a trend to an increase of gray matter volume in the ipsilateral motor cortex; these changes were possibly correlated with motor improvement, because the children increased the use of the affected arm in their daily living activities. Based on these findings, Sterling et al[29] also suggested the use of constraint-induced movement therapy to induce increases in gray matter volume during the development of the nervous system. This reinforces the use of therapies to remodel human brain functioning and to produce motor recovery in patients with motor deficits.

The contralateral cortical activation and ipsilateral cortical deactivation can be explained by transcallosal inhibition; fMRI was used to investigate such inter hemispheric interactions.[35] For this, volunteers performed self-paced sequential finger/thumb tapping for each hand simultaneously with fMRI data acquisition. This experimental design showed that the hand movements produced activation not only in the contralateral sensorimotor cortex, but also in adjacent subcortical regions and even in the ipsilateral cerebellum. Likewise, less activation in the ipsilateral sensorimotor cortex occurred as a response to unilateral hand movements; this was accompanied by decreased blood flow.[35] In our study we found increased gamma band absolute power in frontal cortical areas, especially at the motor area ipsilateral to the movement execution. Thus, the hand immobilization condition may have influenced cortical activation, leading to an important activation of the ipsilateral region in order to perform the movement.

The same pattern of increased gamma band absolute power occurred at central cortical areas (C3, C4, Cz), particularly at the C3 derivation, i.e., in the contralateral motor cortex. This same pattern of activation was shown in a study involving fMRI and self-paced sequential finger/thumb movements;[35] the C3, Cz and C4 derivations are related to somesthetic and motor areas that control limb movements, specifically the hands. The C3 derivation corresponds to the motor area of the right hand and the more pronounced increase of gamma band absolute power at this site denotes a greater involvement of the motor area contralateral to the elicited movement. This finding is confirmed by Muthukumaraswamy,[24] who stated that gamma oscillations in the motor cortex occur primarily at the contralateral hemisphere to the limb that executes the movement. Moreover, the task of this study involved repetitive movements of the index finger, which can be reported to a set of four experiments which showed that gamma oscillations are most likely triggered by broader movements and by the first movement of a repetitive sequence. This same set of experiments showed that the region anterior to the central sulcus in M1 is the source of gamma oscillations.

The findings in the C3, C4 and Cz derivations are justified due to sensorimotor cortex representation, comprising areas linked to motor planning and sensory integrations.[11,36] The Cz derivation corresponds to Brodmann's area 5, which represents the supplementary motor area, which is a useful support for motor pattern elaborations to be sent to the primary motor area.[21] In our study, there was greater activation at C3, 2 sec before and 2 sec after the finger movement in the after-immobilization condition; this indicates more neural binding in this area due to movement planning. In a study with a hemiparetic subject, Machado et al[37] found less activation of the C3 derivation in a hemiparetic subject: they reported that this neural lesion was accompanied by lower values in the gamma band absolute power. We found a higher level of activation at the C3 derivation after hand immobilization because this site represents the motor area responsible for right hand movements and because the subjects presented no neural damage. These results pointed to a relevant activation at central regions due its relation with sensorimotor areas.[6,11,38] Each hemisphere has specifics functions and contributes to motor control differently. The right hemisphere is characterized by use attention and sensory feedback to create spatial references, while the left hemisphere plays a role on movement planning and execution.[39]

The inter-hemispheric differences in gamma band absolute power after immobilization are justified by the ability of the hemispheres to mutually influence their functions through the corpus callosum.[4,40,41] These findings may have a bearing on the applicability of therapies such as transcranial magnetic stimulation, constraint-induced movement therapy, which are widely used in the rehabilitation of stroke[42-44] and cerebral palsy.[45-47] Some studies even indicate the possibility of using the EEG as a central nervous system dysfunctions marker.[48-51] As an example, the specific measurements derived from an electroencephalogram could indicate cortical areas most affected in cases of traumatic brain injury, and may thus be useful for the development of tools for its diagnosis.[48] Furthermore, it would help in the determination of the lesion and the time of return to post-injury sports activities in athletes.[51] Our findings may guide further studies on the use of EEG as the central nervous system dysfunction marker, considering our finding that cortical changes after a relatively short condition of movement deprivation.

CONCLUSION

The current study showed increased gamma band absolute power in fronto-central areas after hand immobilization. Gamma activity promotes the binding of information through different neural sites in order to supply task demands. We have shown that the 48 hours of immobilization were sufficient to alter neural network, diminishing cortical activation in the investigated regions. After immobilization, the movement performance required a higher communication between these areas to allow appropriated planning and movement execution. The increased gamma oscillations can be a cortical therapeutical strategy to solve the effects of movement restriction.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR PARTICIPATION

Bastos VHV, Cagy M, dos Santos RPM, Velasques B reviewed the literature and the final version of the article. Machado D, Teixeira S, Ribeiro P, Machado S developed the project, contributed in work orientation, discussed the data, and reviewed the final draft of the article.

POTÊNCIA ABSOLUTA DA BANDA GAMA REVELA ATIVAÇÃO EM ÁREAS MOTORAS APÓS IMOBILIZAÇÃO DA MÃO

OBJETIVO: O objetivo deste estudo foi analisar mudanças na potência absoluta da banda gamma em áreas corticais motoras, antes e depois de uma condição de imobilização da mão por 48 horas.

MÉTODO: Quinze voluntários saudáveis, com idades entre 20 e 30 anos, foram submetidos a avaliação eletroencefalográfica antes e depois da imobilização, durante a execução de uma tarefa motora desencadeada por um estímulo visual. Uma análise de variância com dois fatores (ANOVA *two-way*) foi empregada para investigar o fator momento (antes e depois do estímulo visual) e o fator condição (antes e depois da imobilização).

RESULTADOS: Um aumento significativo na potencia absoluta da banda gamma foi encontrado após imobilização da mão nos elétrodos (ou derivações) F3, F4, FZ, C3, C4 e Cz.

CONCLUSÃO: Estes resultados revelam que as oscilações na banda gama podem ser uma estratégia cortical para resolver o efeito de menor ativação devido à restrição de movimento. Um melhor conhecimento do funcionamento de áreas corticais motoras após uma condição de imobilização pode orientar estratégias mais eficazes na reabilitação.

PALAVRAS-CHAVE: mão, imobilização, banda gama, EEG

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