Abstract

Hand immobilization has been associated with changes in neural networks of primary somatosensory cortex and primary motor areas. Electrophysiologically, alpha band absolute power may indicate how cerebral cortex processes information. This study aimed to analyze changes in alpha band absolute power on frontal, central, parietal and occipital derivations when hand-movement of subjects was restricted for 48 hours. Fifteen healthy volunteers (20 to 30 years old), were recorded using electroencephalography (qEEG), while exposition to visual stimulus linked to a motor task before and after hand immobilization. Statistical analysis revealed that hand immobilization caused changes in frontal, central and parietal areas of cerebral cortex. In summary, after hand immobilization alpha band absolute power increased in these areas, revealing a lower activation. Contrarily, at C4 there was a decreased alpha band absolute power correlated to more activation. These findings can be due adaptive plasticity to supply less activation at C3, considering the inactivity of right hand due to the immobilization. Further studies are needed to better understand the complex processes involved in this type of task.

Background

Cerebral cortex is a dynamic structure which changes in its organization due a specific condition. This fact can be observed in situations like motor learning and rehabilitation [1]. Particularly, cerebral cortex shows reorganization after a lesion when movements be-
come temporarily or permanently impaired such as in accident that involves immobilization or in a stroke which some cortical functions are affected [2,3]. Understanding these alterations can be useful to propose rehabilitation modalities to restore movements. In this way, hand movements appear to be an interesting source of study due to motor and sensorial representations in the cerebral cortex [4]. Moreover, studies found changes in neural networks in primary somatosensory cortex and primary motor areas (M1) when individuals were subjected limited movements [2,5,6].

Studies had investigated cortical changes occurring after hand immobilization using electroencephalography (qEEG) which consists on useful tool to detect cortical changes associated to especial conditions, such immobilization or using a rehabilitation modality to improve motor function [3,5]. Generally qEEG have been employed to observe changes in cortical activity due tasks or use of some drugs [7,8]. Furthermore, alpha band absolute power may indicate how cerebral cortex processes information, with more mental effort or with less one. Alpha band (8 – 12 Hz) has shown to be strongly associated with attention, cognition, perception and motor process, being related to alertness and cognitive tasks involving attention [9-12]. We are interested on alpha band due to relationship with a relaxation state and with slowness in psycho-physiological process [13]. In addition, the frontal areas are engaged in motivation mechanism, planning to execute voluntary movements, whereas parietais areas are involved in sensorial control mechanism [14-17]. Thus, study involving analysis of the frontal and parietal regions may indicate their functional response in task which requiring specific attention.

Previous studies have been conducted to show the involvement of the cerebral cortex reorganization after motor function limitation condition [5, 18]. But does how cerebral cortex processes visual information linked to a motor task after a condition of immobilization? For this purpose, we seek to answer the following questions: i) Does hand immobilization cause limitation of sensory afferences? ii) Does cerebral cortex changes its activation after 48 hour of the hand immobilization in order to attend the task? Moreover, the activation of occipital areas may be not affected by immobilization, but the activation of sensory motor integration areas may be influenced by absence of movement resulting of immobilization. Another hypothesis is that a competition between stimuli can occurs, in other words, a decreased of attention may happen to the visual stimulus when motor task be required. Thus, our study objectived to analyze the neurophysiological changes occurring in cerebral cortex during visual stimulus exposition linked to a motor task before and after of the hand immobilization. Specifically, this study aimed to analyze changes on alpha band absolute power in the frontal, central, parietal and occipital regions when individuals were subjected at 48 hours of hand immobilization.

**Methods**

**Sample**

The sample was composed of 15 healthy individuals: 04 men and 11 women, with ages varying between 20 and 30 (mean age 24 ± 1.2 years). The individuals were chosen randomly and the recruitment of the volunteers was accomplished thanks to the research announcements posted in different Universities of Rio de Janeiro State. As inclusion criteria, the subjects needed to be right handed, have no mental or physical illness (previous anamnese) and not use any psychoactive or psychotropic substances during the whole time of the study. A detailed questionnaire was applied in order to exclude those individuals who could contaminate the results. Due to hand laterality, the Edinburgh inventory [19, 20] was utilized to identi-
fy the predominance of the participants (right-handed vs left-handed). Consequently, the left-handed individuals were excluded from the experiment. The individuals were instructed to not use tobacco, coffee or alcoholic drinks 10 hours before the test because these substances can influence cortical activation recorded by qEEG [21-23]. The participants received written information about the study procedures and we solicited their signature of the consent form. This study was approved by the ethics committee of Veiga de Almeida University with the number 149,817 in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Tasks and Procedures
The experimental procedure was conducted on a room with acoustic and electrical isolation. During the electroencephalography (EEG) signal acquisition, the lights were dimmed. The subjects sat in a chair with armrest in order to minimize muscle artifact during EEG signal acquisition. In front of the subjects there was a 15-inch monitor on a table. The monitor was turned on only when the subjects executed the task (i.e., flexion and extension of the index finger). Initially, the EEG signal acquisition lasted for 2 minutes (rest) with the monitor off facing the subjects. Then, a sensor to measure acceleration (accelerometer) was coupled on the right index finger; during the visual feedback, the subjects executed the task (i.e., flexion and extension index finger). The accelerometer was connected to the EEG with an additional channel (i.e, channel 21). When the subjects performed the movement, the accelerometer provided a signal for the EEG.

The subjects were instructed to perform the index finger flexion and extension when visual feedback was generated by a random image on the monitor. The subjects executed the task in 6 blocks of 15 trials. In order to avoid muscle fatigue, they rested 3 minutes between each block. Thus, the task has 1 minute each block with 3 minutes interval between blocks which 24 minutes totals task. After completing the task, the monitor was turned off and the subjects were submitted again to EEG during 2 minutes (rest). After EEG recording, a plaster cast was applied on the subjects’ right hand and they kept it on for 48 hours. The plaster cast was applied with hand closed in order to preventing any hand or finger movement. After this period, subjects returned to the laboratory to remove the plaster cast and they were after five minutes plaster cast removed again submitted to the task procedures before hand 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. In addition, two 9-mm diameter electrodes were attached above and on the external corner of the right eye, in a bipolar electrode montage, to monitor artifacts on eye-movements (EOG). Impedance of EEG and EOG electrodes was 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 Data Acquisition (Delphi 5.0) was employed to filter the raw data: notch (60Hz), high-pass of 0.3 Hz and low-pass of 100Hz.

Data processing
To quantify reference-free data, a visual inspection and independent component analysis (ICA) was applied to identify and remove any remaining artifacts, i.e, eye blinks and ocular movements, produced by the task [24]. Data from individual electrodes exhibiting loss of contact with the scalp or high impedances (>10 kΩ) were discarded, and
data from single-trial epochs exhibiting excessive movement artifacts (± 100 µV) were also deleted. ICA was then applied to identify and remove any artifacts after the initial visual inspection. ICA is an information maximization algorithm to blind the EEG signals related to the artifacts [24-26]. Independent components resembling eye-blink 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 reinspected 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 4s periods (the selected epoch started 2s before and ended 2s after visual stimulus).

**Selected derivations and frequency band**

Derivations in the frontal region (Fp1, Fp2, F3, F4, F7, F8 and Fz) were selected due to their functional relationship with motivation, planning and motor program [26]. Other derivations were selected due to their relationship with motor act (C3, Cz and C4) [27]. The derivations representing parietal region (P3, Pz and P4) were chosen due to their functional relationship with sensorimotor integration [28]. Moreover we selected derivations in the occipital region (O1, Oz and O2) were chosen because its association with visual function that was necessary to respond the stimulus. Alpha band was chosen because shows relationship with relaxation state or slowness of psychophysiological processes and can indicates mental effort of cerebral cortex in processes information. Furthermore, alpha band have been associated with attentional suppress per visual and somatosensory systems [29-32]. Beside, absolute power was elected because corresponds to total energy of a derivation on a certain region at different frequency bands [33].

**Statistical Analysis**

The statistical design allowed the examination of functional cortical action before and after 48 hour of hand immobilization. Areas in each region, with respective regions related to sensory, motor execution, and integrative or associative functions (i.e, electrodes Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz) were selected. Data were standardized and normalized into values of absolute power using homocedasticity in a natural logarithmic test (LogN) in order to approximate values to a normal distribution [34-35]. Thus, two factorial designs were employed for alpha band with factor moment (before vs after visual stimuli) and factor conditions (before vs after 48 hours hand immobilization). The interaction was investigated with one-way ANOVA with factor condition for each EEG acquisition. The significance levels were set at p ≤ .005. These analyses were conducted utilizing the SPSS for Windows version 18.0 (SPSS Inc, Chicago, IL, USA).

**Results and discussion**

The results of two-way ANOVA showed main effect for condition (i.e, before immobilization vs after immobilization) at Fp2 derivation [F(1,3812) = 4.032; p=0.045]. The statistical analysis showed an increased on alpha band absolute power after hand immobilization, leading to believe that immobilization influenced alpha oscillation. A one-way ANOVA was used in order to investigate interaction. Thus, the qEEG analysis at Fp1 derivation showed interaction for condition (i.e, before immobilization vs after immobilization) and moment (i.e, before visual stimulus vs after visual stimulus) [F(1,3841)=5.430; p=0.02] (Figure 2). Our statistical results of one-way ANOVA for interaction was [F(3,3844)=7.924; p=0.001]. Post hoc Bonferroni test showed signifi-
A significant difference between pre and post hand immobilization. There was an increased on alpha band absolute power comparing pre immobilization before stimulus (mean 1.42 ± 1.03) with post hand immobilization after stimulus (mean 1.59 ± 1.11). Similarly, an increased on alpha band absolute power

Figure 1: A) An interaction for condition (before immobilization in first visit versus after immobilization in second visit) and moment (before versus after visual stimulus) in left fronto polar cortex (Fp1) (p<.001). B) Mean and SD indicate main effect for condition (before immobilization in first visit versus after immobilization in second visit) observed in right fronto polar cortex (Fp2) (p=.045).

Figure 2: a) Main effects for factor treatment observed in the left central cortex (C3) derivation by mean and SD (p < .001); b) Main effects for factor treatment observed in the left parietal cortex (P3) derivation by mean and SD (p < .001).
occurred when compared pre immobilization after stimulus (mean $1.37 \pm 0.99$) with post hand immobilization after stimulus (mean $1.59 \pm 1.11$). Probably the immobilization influenced the activation on frontal polar areas (Figures 1A and 1B).

In central region, the two-way ANOVA showed main effect at [C3 $F(1,3585)=4.642; p=0.031$] (Figure 2A) and [C4 $F(1,3647)=12.103; p=0.001$] (Figure 2B) for condition (before vs after immobilization). An increased on alpha band absolute power was seen at C3 derivation between before (mean $0.577 \pm 0.010$) and after (mean $0.608 \pm 0.010$) hand immobilization. The opposite occurred at C4 derivation which showed before (mean $0.555 \pm 0.009$) and after (mean $0.510 \pm 0.009$) hand immobilization a decreased on alpha band absolute power. These findings demonstrated that C3 and C4 derivations act differently when individual are submitted the hand immobilization. Moreover, C4 derivation may went more activate because 48 hours of the hand immobilization, subjects used more the left hand due immobilization of the right one.

The two-way ANOVA implemented to analyses parietal region revealed significative difference at P4 derivation, $[F(1,3296)=5.072; p=0.024]$ (Figure 3). An increased on alpha band absolute power occurred after hand immobilization. In contrast to the results found in frontal, central and parietal areas, there were no significative differences on occipital region, O1 ($p=0.51$), O2 ($p=0.83$) and Oz ($p=0.70$) showing that this region was not influenced by hand immobilization according us expected because the immobilization may be more associate between sensory and motor than visual function.

This study was conducted in order to analyze the neurophysiological changes occurring in cerebral cortex during an exposition to visual stimulus linked to a motor task before and after an immobilization condition of the hand. Thereby, the study aimed to analyze changes in alpha band absolute power on frontal, parietal and occipital derivations when individuals are subjected to restriction of movement for 48 hours. Moreover, was hypothesized that after 48 hours of hand immobilization cortical changes occur in the ipsi- and contralateral hemisphere, was expected find changes in absolute power of the derivations studied, because it represents a fraction of the signal power concentrated in alpha band.

We observed that alpha band absolute power increased at Fp2 derivation after hand immobilization. Thus, the frontal region, in special, the frontopolar cortex seems be activated during task which involving planning, problem solving and reasoning [36]. Studies indicate that frontopolar cortex has particular importance in cognitive function, highlighting its role in to hold goals in mind while exploring and processing secondary goals, process named multitasking [37-40]. Study with functional magnetic resonance imaging revealed that bilateral regions in the frontopolar prefrontal cortex are selectively activated when subject hold in mind goals while simultaneously process secondary goals (dual-task), this process usually required in planning and reasoning [37].
The task proposed in our experimental design can be understood like dual-task because required processing visual information and performance the motor task (i.e., flexion and extension index finger). The increased on alpha band absolute power at Fp2 derivation after hand immobilization may indicated lapse in attention to the performance of dual-task [41], and may reflect the fact that the task does not require functions linked to the frontopolar region, i.e. planning, problem solving and reasoning [36]. The activity increased on the alpha band usually happen in subjects who has attention deficit hyperactivity disorder [12], but it is worth noting that volunteers of this study were assessed by previous anamneses which objective consisted in exclude those who had mental illness. Moreover, Fp2 derivation is linked to the left corporeal side that was neither submitted to the immobilization nor involved in motor task. It may be consider that volunteers of this study were right handed, so a possible change occured at Fp1 derivation due to its relationship with right corporeal side that was involved by hand immobilization and recruited in motor task.

The interaction found at Fp1 derivation for condition (before vs after immobilization) and moment (before vs after visual stimulus) evidenced an increased on alpha band absolute power, i.e. alpha band absolute power was higher after visual stimulus and after immobilization than when compared to before visual stimulus and before hand immobilization (Figure 2). Probably, before immobilization and visual stimulus the neural circuitry was in readiness to identify visual stimulus and execute motor task. Thus, we understood that after hand immobilization and visual stimulus, the increased on alpha band absolute power indicates that this circuitry became less activated [42]. The immobilization may have caused less afference to right hand and absence of movement made the region less active. A research involving work memory indicated prefrontal areas involvement in visual-spatial tasks that required considerable level of difficulty, and when this occurred, there was less activation in alpha band [43]. The involvement of prefrontal areas in executive functions is reflected by a decrease of alpha. In our study, the finding of high values in alpha band in frontal areas may denote a feature of the task that not required executive functions (easiness task).

Furthermore, visual stimulus was presented for 15 times in each block, totalizing 90 stimuli per six blocks. Like this, after several presentations of the visual stimulus, the attention level decreased because the subjects knew what would be presented to them, especially in condition after immobilization because they had knowledge about the experimental procedure. The increased on alpha band absolute power reflect habituation mechanism, which is viewed in some studies like a process in which the response to a repetitive stimuli tends to decrease [44-45]. Besides, researches indicate that combined tasks leading to suppression of neural circuitry activity accountable for execution of the first task while areas involved in the second one showing consequently increase in its activation [46-47]. Perhaps, in our study, the performance to the motor task (flexion and extension of the index finger) suppressed the neural circuitry activity at region of Fp1 derivation during the identification of the visual stimulus according Foxe & Simpson (2005) that using high-density mapping of human event-related potentials examined the brain activity associated with selective information processing when subjects were cued on a trial-by-trial basis to perform a discrimination in either the visual or auditory modality. These authors suggest that when individuals are required to switch rapidly from execution of one task to another, goal-related task networks and attentional mechanisms are engaged to reconfigure task-specific networks, suppressing activity within circuits responsible for performance of the old task and amplifying preparatory neural processes for the anticipated novel task.

We observed increased at C3 derivation after hand immobilization probably, due to the relation-
ship between right hand with C3 derivation which is involved with motor act [48]. Thereby, the right hand immobilization may diminished afference resulting in less activation in the central area which showed high values in alpha band after hand immobilization. Indeed, ours findings are according studies which indicate that occur plastic changes on motor cortex at the area of hand’s representation when occur hand immobilization [3]. Several studies indicate an adaptive plasticity per less representation of the cortical area after amputation or movement restriction [49-51]. The literature shows that chronic reduction on neuromuscular activity caused by immobilization results in muscular atrophy, weakness and damage to the motor function linked to changes on cortical excitability [49]. A sensorimotor restriction causes damage to motor function due structurally and functionally reorganization of sensorimotor cortex [3]. Study involving microscopy verified that 14 days of immobilization of hind feet of rats are sufficient to cause decrease on dendritic spine length and others morphological changes associated to reorganization of motor cortex in response to the decrease on sensorimotor function [50]. In this sense, we highlight that immobilization can alter neural networks responsible to the motor act.

Corroborating our findings, a study involving saccadic task also found increase on alpha band absolute power at C3 after visual stimulus. The authors correlate this finding to the automation of the task; in others words, high levels of attention were less necessary to execute the task [52]. In our study, we understood that before hand immobilization functioned as training. In this case, at condition after hand immobilization the subject knew the task which promoted less attention lever for its execution. Furthermore, the task repetition caused less recruitment in the central area, which may signalize learning of the task [52].

Contrary to the others derivations, at C4 alpha band absolute power decreased, leading to believe that this region was more activated. Thus, the inactivity of right hand due to hand immobilization caused adaptive plasticity at C3 and demanded highest mental effort of corresponding contralateral area to attend the task [42]. Studies have shown that intact neurons can compensate function loss of neurons injured. Thereby, hand immobilization had created diminish of afference reflecting in less activity at C3 area which to be compensate required participation of the contralateral homologous cortical area (C4) [53-54]. This compensation seems occur via corpus callosum which has been observed on studies involving patients, healthy subjects and even chimpanzee [55-56].

Studies involving functional magnetic resonance imaging revealed that amputation and deafferentation induce changes on sensorial (S1) and motor (M1) cortex [57-59]. Likewise, Viaro et al. (2014) [51], using intracortical microstimulation in adults rats, found cortical changes due to movement restriction. Traditionally, it was knew that cortical area responsible per sensorimotor behavior of amputated segment was transferred to functionalities of neighboring regions [60]. Simões et al. (2012) [57]; related a reconfiguration beyond this one, they found changes on cortical representation of the intact segment (not amputated). For example, in amputated subjects, tactile stimulation on intact foot induced high activation on S1 and M1 ipsilateral when compared with subjects no amputated (control). This finding according to our findings of highest cortical activation ipsilateral to the hand immobilized. The hand immobilization per 48 hours can function as a deafferentation mechanism [51].

In parietal region was seen an increased on alpha band absolute power at derivation P4. Anatomically, the control of spatial attention is localized to dorsal frontoparietal regions [61]. Recent evidences denote an important role of the posterior parietal cortex on attention-related modulation of visual cortex activity [62]. Study used repetitive Transcranial Magnetic Stimulation observes that it involved
interference of intraparietal cortex during allocation of spatial attention which causes disruption of anticipatory (pre-target) EEG de-synchronization of alpha rhythms (about 8–12 Hz) in occipito-parietal cortex, mainly in the hemisphere contralateral to the locus of attention [62]. Moreover, the parietal area is associated to the function of interpretation and integration informations [28], being P4 derivation correspondent to the left side of the body [63]. In this way, the absence of requirement in interpretation and integration informations came from left side of the body resulted in increase of the alpha band absolute power, reflecting a state of relative neural inactivity on the analyzed region [42]. Complementarily, visual stimuli are secondarily processed in parietal areas [64], then an increased on alpha band absolute power in this region indicate that the stimuli did not required high levels of attention to be processed. In fact, the task can be considered easy to execute, once required only index movement (flexion and extension) as response to the visual stimulus who was presented.

Lastly, ours study find no significative difference in occipital region, as it was expected due no influence of immobilization to the function of the occipital cortical area which is more likely associated to process visual information [64]. In several clinic situations, the visual function have served as compensating sensory deficits, i.e. diabetic patient frequently uses vision to examine and then choses secure surroundings avoiding possible lesions on lower extremities affected by neuropathy [65]. Therefore, front to a situation of movement restriction, vision can assists the lack of afference linked to the immobilization and consequent no interaction of the segment in the environment. Our finding of no significative difference on alpha band absolute power at occipital region between before and after hand immobilization indicates that this area kept exerting its function on similar way, despite immobilization. Therefore, our findings in occipital region demonstrate the no influence of hand immobilization on visual areas is a positive aspect because motor damage caused by immobilization can be, somewhat, minimized by visual attention [65]. Beyond that, the visual function diminished is associated with worst motor performance. A study involving force plate sway and gait velocity showed decrement in performance on subjects who had loss of vision [66].

Conclusions
The findings of our study allow concluding that there are cortical changes when a visual stimulus linked to a motor task is presented to the subject. Hand immobilization can causes limitation of sensory afferences and this lead to changes in cerebral cortex activation after 48 hours. Particularly, the study found changes on alpha band absolute power that denote a response to immobilization per 48 hours. At C4 derivation, lower values of alpha band absolute power indicate highest activation at this region as a compensatory strategy to execute the task after immobilization. The increased on alpha band absolute power at Fp1, Fp2, C3 and P4 indicate less activation of these regions after immobilization and may be due to 1) features of the task (easiness of execution); 2) learning of the task from the condition before immobilization; 3) habituation caused by repetitive stimuli; 4) memory lapse resulting of competition between visual stimulus and motor task; and 5) decrease of sensory-motor function generated by immobilization. In future studies be convenient analyze alpha band absolute power including rest as a third condition. Additionally, to before and after immobilization. Further studies are needed to better understand the complex processes involved in this type of task.

Competing interests
None of the authors have actual or potential conflict of interest including any financial, personal or other
relationships with other people or organizations that could inappropriately influence, or be perceived to influence, our work.

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Authors’ contributions

DM, ST, BV, PR, and OAC designed, conducted the experiment and drafted most of the manuscript. DM, VHVB, ST, MC, ASC, JSP and SM performed the experiment, the literature review and the drafting of the manuscript. All authors read and approved the final manuscript.

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