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Antonio Alejandro Villasana De Abreu

Dissertação para obtenção do Grau de Mestre em
Medicina

fevereiro 2024



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(mestrado integrado)

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Declaração de Integridade

Eu, Antonio Alejandro Villasana De Abreu, que abaixo assino, estudante com o número de inscrição 41122 do Mestrado Integrado em Medicina da Faculdade ciências da saúde, declaro ter desenvolvido o presente trabalho e elaborado o presente texto em total consonância com o **Código de Integridades da Universidade da Beira Interior**.

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Universidade da Beira Interior, Covilhã 25 /02 /2024



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Dedication

*“Gloria al bravo pueblo
Que el yugo lanzó
La Ley respetando
La virtud y honor
¡Abajo cadenas!
Gritaba el Señor
y el pobre en su choza
libertad pidió
A este santo nombre
tembló de pavor
el vil egoísmo
que otra vez triunfó.
Gritemos con brío:
“¡Muera la opresión!”
Compatriotas fieles,
la fuerza es la unión;
y desde el Empíreo
el Supremo Autor,
un sublime aliento
al pueblo infundió.
Unida con lazos
que el cielo formó
la América toda
existe en nación;
y si el despotismo
levanta la voz,
seguid el ejemplo
que Caracas dio.”*

- Gloria al Bravo Pueblo, 1816

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To my volunteers, who slept less for science and for me.

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Resumo

Introdução: A fadiga é um sintoma descrito como falta de energia e exaustão podendo ser física ou cognitiva. Atualmente, a sua medição depende exclusivamente de escalas de percepção subjetiva. A estimulação transcraniana magnética e o protocolo de estimulação *theta burst* continua (cTBS) surge como um método não invasivo usado para explorar e modular funções cerebrais e o processamento cognitivo. Assim, esta investigação tem como objetivo o estudo e quantificação de distintos parâmetros biológicos que possam associar-se à fadiga e avaliar os diferentes efeitos que a cTBS têm em indivíduos fatigados.

Materiais e métodos: Realizou-se um estudo “crossover” em voluntários saudáveis – amostra com 7 voluntários (6 indivíduos do sexo feminino e 1 do sexo masculino); no teste A procedeu-se à realização de exercício aeróbio moderado e redução do sono em 20% do tempo habitual e no teste B não se procedeu a alterações do estilo de vida. A fadiga foi avaliada através da escala de Chalder e foram realizados: o teste de fluência verbal, teste de reação visual, medição da oximetria cerebral e avaliação de potenciais evocados cognitivos (P300), em ambos os testes, antes e após a aplicação da cTBS. Procedeu-se um follow-up de 4 semanas da percepção de fadiga, com a escala de Chalder. Para a análise estatística recorreu-se ao teste de Friedman e, quando significativo a 5%, as comparações aos pares foram obtidas com o teste de Dunn com correção de Bonferroni. As correlações foram medidas usando o coeficiente de correlação de Spearman.

Resultados: Como esperado, no teste A, os voluntários foram considerados fatigados de acordo com a escala de Chalder, mas no teste B não; contudo a diferença não foi estatisticamente significativa. No teste de fluência verbal, ambos os grupos A e B mostraram melhor performance depois da cTBS, sem diferença significativa entre ambos. A cTBS originou a lentificação da latência do P300, potencialmente de forma mais pronunciada no grupo do teste A.

Conclusão: Apesar de uma possível diferença entre os grupos A e B nos resultados do P300 após cTBS associada ao grupo fatigado, esta não foi significativa. Assim, no presente estudo não foi possível concluir que a fadiga pode ser medida de forma objetiva. Vários fatores limitantes, nomeadamente o número de sujeitos, podem ter contribuído para isso.

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Palavras-chave

Fadiga cognitiva; fadiga física; estimulação magnética transcraniana; TMS; cTBS; teste de fluência verbal; teste de reação visual; NIRS.

Resumo alargado

Introdução

A fadiga é um sintoma descrito como falta de energia e cansaço e está associado à incapacidade de realizar atividades do quotidiano e pode ser cognitiva, física ou mista. A fadiga física pode ocorrer devido a disfunção na geração de força. Já a fadiga cognitiva pode ser definida pela diminuição da capacidade cognitiva devido à demanda mental sustida.

A fadiga está relacionada com inúmeras patologias e é uma fonte importante de erros potencialmente graves na execução de tarefas moderadamente complexas. Por exemplo, de acordo com a NASA, 21% dos acidentes de aviação são relacionados com a fadiga. Já no campo da saúde, 61% dos médicos e 80% das enfermeiras reportam *burnout*.

Atualmente, a medição dos níveis de fadiga depende exclusivamente de escalas de perceção subjetiva. Um dos objetivos desta investigação passa pelo estudo e quantificação de distintos parâmetros biológicos que se possam associar à mesma, como por exemplo o teste de fluência verbal, a oximetria cerebral e o teste de reação visual.

Já a estimulação magnética transcraniana surge como um método não invasivo usado para explorar e modular funções cerebrais, e o protocolo de estimulação *theta burst* continua, utilizado no presente estudo, permite avaliar os diferentes efeitos que a mesma têm em indivíduos não fatigados versus fatigados.

Métodos

Realizou-se um estudo “crossover” em voluntários saudáveis – amostra com 7 voluntários (6 indivíduos do sexo feminino e 1 do sexo masculino). Foram excluídos indivíduos com elementos metálicos no crânio, com epilepsia, com diversas patologias do sistema nervoso, cardíacas, respiratórias e / ou psiquiátricas, neurocirurgia prévia, gravidez, medicação que diminua o limiar convulsivo, alcoolismo, uso de drogas ilícitas. Foram também excluídos indivíduos com mais de 30 anos ou menos de 18 anos, indivíduos não destros, e indivíduos que não durmam entre 6-11 horas em média por dia, ou que pratiquem mais do que 300 minutos de exercício aeróbio por semana ou mais de 3 sessões de treino de força.

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No teste A procedeu-se à realização de exercício aeróbio moderado e redução do sono em 20% do tempo habitual e no teste B não se procedeu a nenhuma alteração do estilo de vida. A fadiga foi avaliada através da escala de Chalder e foram realizados os seguintes testes: teste de fluência verbal, teste de reação visual, medição da oximetria cerebral e avaliação de potenciais evocados cognitivos, em ambos os dias (testes A e B), antes e após a aplicação da estimulação magnética transcraniana. Foi realizado um follow-up da percepção de fadiga, com a escala de Chalder, dois dias e quatro semanas após o dia do estudo.

Para a análise estatística recorreu-se ao teste de Friedman e, quando significativo a 5%, as comparações aos pares foram obtidas com o teste de Dunn com correção de Bonferroni. As correlações foram medidas usando o coeficiente de correlação de Spearman.

Resultados

A amostra foi constituída por 7 indivíduos (6 do sexo feminino e 1 do sexo masculino) com idades entre os 22 e os 28 anos. A mediana de horas dormidas no teste A foi de 6 (o que equivale a uma mediana de 80% do sono habitual) e no teste B de 6 horas também (o que equivale a uma mediana de 88% do sono habitual).

No teste A, os voluntários foram considerados fadigados de acordo com escala de Chalder (pontuação mediana igual a 4), mas no teste B não; contudo a diferença encontrada entre os grupos não foi estatisticamente significativa.

No teste de fluência verbal, ambos os grupos A e B mostraram melhor performance depois da cTBS, sem diferença significativa entre ambos. Apesar do resultado ter sido melhor no teste B, a diferença não foi estatisticamente significativa. Relativamente à oximetria cerebral apenas se observou que houve um aumento da mediana após aplicação da cTBS no teste A e uma diminuição no teste B, mas sem significância estatística. O tempo de reação visual também demonstrou ser inferior no teste B e após a cTBS, contudo, sem diferença estatística.

No que toca aos potenciais evocados, as latências do P300 foram significativamente lentificadas após cTBS em ambos os grupos, existindo uma diferença marginalmente significativa devido a latências superiores no teste A. Já no que toca à amplitude do P300 não se registaram diferenças significativas nem assimetrias relevantes entre o teste A e B.

Conclusão

Conclusão: Apesar de uma possível diferença entre os grupos A e B nos resultados do P300 após cTBS associada ao grupo fatigado, esta não foi significativa. Ainda, apesar de possíveis diferenças entre os grupos A e B nos distintos exames aplicados para o estudo da fadiga, não foram observadas diferenças significativas. Pelo que, no presente estudo não foi possível concluir que a fadiga possa ser medida de forma objetiva. Vários fatores limitantes podem ser apontados, nomeadamente o número de sujeitos, os exames aplicados, o quotidiano dos voluntários, entre outros.

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Abstract

Introduction: Fatigue is a symptom described as the lack of energy and exhaustion and can be physical or cognitive. Currently, its measurement depends exclusively on subjective perception scales. Transcranial magnetic stimulation and the theta burst stimulation protocol (cTBS) appears as a non-invasive method used to explore and modulate brain functioning, may influence cognitive processing. Therefore, this investigation has as objective the study and quantification of different biological parameters that may be associated with fatigue and evaluate the different effects of cTBS in fatigue individuals.

Methods and materials: A “crossover” study was carried out with 7 healthy adult volunteers (6 females and 1 male). In test A was proceeded to the realization of moderate aerobic exercise and reduction of sleep by 20% of the usual amount, and in test B was not proceeded to lifestyle changes. The fatigue was measured using Chalder scale and were performed verbal fluency test, visual reaction test, measurement of cerebral oximetry and evaluation of evoked cognitive potentials (P300), before and after the application of cTBS. It was proceeded to a follow-up of 4 weeks of the fatigue perception. For statistical analysis, the Friedman test was used and, when significant at 5%, pairwise comparisons were obtained with the Dunn test with Bonferroni correction. Correlations were measured using Spearman's correlation coefficient.

Results: As expected, on test A, volunteers were considered fatigued according to chalder scale, but in test B they were not; however, the difference was statistically non-significant. In the verbal fluency test, both A and B groups shown better performance after cTBS, with no significant difference between them. cTBS slowed P300 latency, potentially more pronounced on the test A group.

Conclusion: Despite a possible difference between groups A and B in P300 results after cTBS in the fatigued group, this was not significant. Therefore, in the present study it was not possible to conclude that fatigue can be measured objectively. Several limiting factors, namely the number of subjects, may have contributed to this.

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Keywords

Cognitive fatigue; physical fatigue; transcranial magnetic stimulation; TMS; cTBS; aerobic exercise; verbal fluency test; visual reaction test; NIRS.

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Acronym list

%HRres – percentage of the heart rate reserve

CS – Chalder Scale

CSP - Chalder Scale punctuation

cTBS – continuous TBS

EP – evoked potential

ERP – event-related potential

HbO₂ – oxy-hemoglobin

HHb – deoxy-hemoglobin

HR – heart rate

HR_{max} – maximal heart rate

HR_{res} – heart rate reserve

Max – maximum

Min – minimum

NIRS – near infrared spectroscopy

NW – new words

RT – auditive reaction test

rTMS – repetitive TMS

s2P – sleep-2-peak

sd – standard deviation

TBS – theta burst stimulation

TMS – transcranial magnetic stimulation

VFP – verbal fluency test performance

VFT – verbal fluency test

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Chapter 1

Introduction

Fatigue can be defined as “a symptom that cannot be relieved by the usual strategies of restoring energy, and that impairs, to a variable degree, the individual’s ability to carry out his or her usual daily activities”, described as “listlessness, lack of energy, exhaustion, tiredness, early fatigability, sleepiness, a tendency to fall asleep during the day, physical weakness, or a feeling of running on empty”, and it is the main or a secondary reason for 10–20% of patient consultations in primary care. (1)

Fatigue can be either physical or cognitive. Physical fatigue occurs due to dysfunction of at least one process related to the generation of force by the contractile proteins. (2) Cognitive fatigue can be defined as the diminishing cognitive capacity due to sustained mental demand. (3) Moreover, there is an acute inverse relation between the exercise demand and performance on memory tasks. (2)

A huge number of medical disorders, such as mental illness, cancer, anemia, endocrine disorders, infectious and liver diseases, chronic somatic diseases, sleep disorders can cause fatigue, either physical or cognitive. (1) Fatigue can also occur associated with sleep problems: since the duration, continuity, and depth of sleep without circadian rhythm disruption is necessary to achieve high levels of attention and cognitive performance as well as to prevent the development of different pathologies. (4) According to NASA’s Aviation Safety Reporting System, 21% of reported aviation incidents, and one-third of all the incidents, errors or problems reported to UK Confidential Human Factors Incident Reporting Programme are fatigue-related. (5) A survey to professional drivers in Poland showed that a majority (89%) declared suffering from fatigue at work, however, those with more age-related experience reported less fatigue. (6) Healthcare professionals are no exception. 61% of doctors reported feelings of burnout as well as 80% of nurses, relating it several clinical mistakes. (7)

Fatigue can be subjectively measured using questionnaires, as the Chalder Scale (CS), which is a multidimensional scale assessing both mental and physic fatigue. (8) Levels of sympathetic nerve activity and parasympathetic nerve activity are proportionally and inversely related to Chalder Scale punctuation (CSP), respectively. (9) One of the major problems is that fatigue per se is a subjective feeling and although we recognize the Chalder

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scale as reliable, its results could be falsified for many reasons, making them untrustworthy if the objective is to use them in day-to-day situations. Therefore, the ideal solution would be to find fatigue parameters or patterns applicable in everyday life, without great costs and with the least number of subjectivities.

Verbal fluency test (VFT) can be used to distinguish between the existence or presence of cognitive impairment (10) and is closely linked to working memory, motivation and attention (11). We may expect a negative relation between fatigue and verbal fluency (12); however, levels of anxiety can obscure this relation because verbal fluency test performance (VFP) scores are improved in individuals with higher levels of stress. (12)

Cerebral oxygenation can also be quantified to assess fatigue. For instance, during a driving task, oxygen consumption increases and induces an increase in cerebral blood flow, which causes a raise in oxy-hemoglobin (HbO₂) accompanied by a decrease in deoxy-hemoglobin (HHb). Prolonged regional neural activity causes a reverse behavior at the end of a driving task – rise in HHb and drop in HbO₂, that can be non-invasively measured by near infrared spectroscopy (NIRS). (13)

Transcranial magnetic stimulation (TMS) is a non-invasive method used to explore brain function and to help diagnose and treat some disorders, namely depression. It is based on the application of magnetic fields that generate changes in neurons and modify their excitability. (14) Theta burst stimulation (TBS), a repetitive TMS (rTMS) protocol, modulates cortical excitability in less than 5 minutes, being able to induce immediate and prolonged functional and structural plasticity. Continuous TBS (cTBS) is an inhibitory application, which can increase the reaction time, but also task-specific connectivity demonstrating fast, adaptive, and functional cerebral reorganization just after one session. (14)

The auditory P300 is a “positive waveform that reaches a maximum at approximately 300 milliseconds after stimulus onset”. P300 is a component of event-related potentials (ERP). Even though its amplitude is related to the “amount of cognitive effort” allocated, larger amplitudes are “associated with fewer errors by children performing in the oddball paradigm task”. P300 latency might be coupled with “speed and efficiency of information processing”. (15) In line with what has been observed in other studies, this indicates that cognitive fatigue, and number of hours awake are positively related to latency and negatively to amplitude of the P300 evoked potential. (16) P300 most likely results from glutaminergic neurotransmission, indirectly affected by GABAergic transmission (reduces amplitude and enhance latency when given sedating GABAergic drugs) and neuromodulated by the cholinergic system (enhance amplitude and reduce latency when given scopolamine). (17)

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Previously, the use of cTBS delayed P300 latency in both hemispheres, being possibly effective in modulating dopaminergic and glutaminergic connection. (18) If applied to the left hemisphere cTBS seems capable to bilaterally reduce prefrontal cortex oxygenation. (13)

1.1 Main Objectives

This study aims to induce physical tiredness and lack of sleep, in healthy students and evaluate and quantify their associated fatigue.

The first objective of our research is to evaluate the different effects of cTBS in tired and non-tired students. The second objective is to examine the degree to which cTBS can influence fatigue in these subjects. The third purpose is to analyze the results and conjugate them to see which objective and subjective tools or combination of tools seem most effective measuring objectively fatigue.

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Chapter 2

Materials and methodology

2.1 Study type

Our research is a quantitative prospective crossover study. Participation in the study was free and informed, with volunteers signing an informed consent (Appendix I) prior to the procedure. The exclusion and inclusion criteria were explained, as well as the entire procedure and possible side effects. Furthermore, anonymity was ensured both in the collection and processing of data.

The initial project in which this study is part (CE-FCS-2011-001) received a positive opinion from the Ethics Committee of the Faculty of Health Sciences (Annex I).

2.2 Sample selection

Contact and selection of volunteers were carried out between March and September of 2022. Recruitment of participants was carried out via social networks aiming students of the Integrated master's degree in Medicine at the Faculty of Health Sciences of the University of Beira Interior.

The students who expressed their willingness to participate in the study were invited to fill out a questionnaire that evaluated them according to inclusion and exclusion criteria (Appendix II).

Inclusion criteria

1. Right-handed students
2. Students between 18-30 years

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1. Individuals with metallic elements in the skull region (excluding the oral region).
2. Personal history of seizures or epileptic seizures.
3. Diagnosis of neurological, cardiac, respiratory, or psychiatric pathology.
4. Pathology that causes increased intracranial pressure.
5. Previous neurological surgery.
6. Confirmation or suspicion of pregnancy.
7. Individuals on medication that lowers the seizure threshold, without concomitant anticonvulsant intake.
8. Alcoholism or use of illicit drugs.

Because sleepiness and exercise may alter some of the exams applied and are somehow related with fatigue, subjects that don't sleep, on average, between 6-11 hours (19) are also excluded from the study, as well as those subjects that exercise more than 300 minutes of aerobic physical exercise or 3 sessions of strength exercise, per week. (20)

In both Test A and B, the consumption of tea, coffee or other drinks that contain stimulant substances (caffeine, theine, taurine, among others) in the 12 hours before taking the exams was also considered an exclusion criteria. Also, the consumption of alcohol in the 24 hours before the exams was considered an exclusion criteria.

After being included in the study, every subject received a random identification code. Data was anonymized and those responsible for evaluating each volunteer in relation to the exclusion criteria only had access to the code.

Scheduling was carried out over the telephone and volunteers were instructed about certain conditions relevant to the study, depending on the test they were going to perform (test A or test B – see in the procedure).

2.3 Procedure

Every subject was submitted to two different protocols of tests which were:

1. Test A
 - a. Reduction of sleep time by 20% of the mean usual sleep time
 - b. Moderate intensity of brisk walking during 15 minutes before the tests and TBS, preceded by the measurement of the baseline heart rate (HR)

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After exercise, a pause of 10 minutes was done or until the baseline HR was reached.

2. Test B – 3 months after test A
 - a. No additional lifestyle changes were made before the tests and TBS were performed.

For simplification, the day of test A will be referred as simply as test A and the day of test B will be referred as test B.

2.4 Proceeded exams/tests and theta burst stimulation

All participants went through the same battery of exams/ questionnaires/ tests /stimulation:

1. Measurement of ambient temperature, measurement of body temperature and characterization of exercise intensity by HR
2. Chalder Scale – initially and as follow-up 2 days and 4 weeks after
3. Verbal fluency test (in test A, after exercise)
4. Reaction test (in test A, after exercise)
5. NIRS
6. P300
7. Inhibitory TBS
8. Exams 3 to 6 were applied both before and after the inhibitory TBS.

Before starting the session, volunteers were invited to read the Informed Consent prepared in accordance with the Declaration of Helsinki (Appendix I). The signing of the informed consent was accompanied by a brief oral explanation about the procedure and possible side effects of the stimulation, aiming to clarify any doubts.

All volunteers were instructed to contact the research team if any adverse effects were to be reported within 48 hours post-stimulation.

2.5 Methods

2.5.1 Heart rate

HR was measured using a Polar® Unite Fitness Watch, placed on the left arm. The basal volunteer’s HR considered was the value measured before the beginning of the exercise. Because Fox formula ($220 - \text{age}$) showed to assure adequate exercise intensity (21), it was used to predict the maximal heart rate (HR_{\max}) and calculate the heart rate reserve (HR_{res}).

Table 1 – The percentage of the heart rate reserve (%HRres) required to considerer an exercise as moderate.

| Exercise intensity | % HR _{res} |
|--------------------|---------------------|
| Moderate (22) | 40-59% |

2.5.2 Chalder scale

Scales that intend to assess fatigue are unidimensional or multidimensional. Chalder scale is a multidimensional scale allowing for a broader and more complete assessment of fatigue, both mental and physic. It allows to distinguish between subjects with or without fatigue measured with Checklist Individual Strength. (8)

Every item is valued from 0-3, relating with the intensity of fatigue. Every score equal to 0 or 1 is converted to 0 points, and 2 or 3 to 1 point. An individual with a cumulative mark of at least 4 is considered fatigued. (23)

2.5.3 Visual reaction test

It is known that “after a high intensity effort, physical and mental fatigue may appear” which affects the ability to react quickly. Inducing a short period of physical fatigue is sufficient to augment the visual reaction time in athletes. (24) The app used to assess visual reaction time was sleep-2-peak (s2P), validated in 2016; it aims to assess changes in the alertness of individuals after long periods of wakefulness or sleep deprivation. (25) The assessment consists of a 3-minute test in which the volunteers, while holding the cell phone with their

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left hand, presses the screen as quickly as possible with their right index finger as soon as an image of the Sun appears. The result is the subject's visual reaction time average. (25)

2.5.4 Verbal fluency test

The assessment consisted in a 3-minute test (1 minute per letter) in which it was asked to the volunteers to say as many words as possible starting with M, P and R, except augmentatives, diminutives, and personal nouns. (10) It was made before and after TMS.

2.5.5 Near infrared spectroscopy

Cerebral oxygenation values were obtained with the EQUANOX™ device, Model 7600 Regional Oximeter System with EQUANOX Classic™ Sensor, Model 8000CA. Two sensors were used placed in the frontal region, corresponding to the left and right cortex, approximately 5 cm above the eyebrow line. Both prior and after stimulation, measurements were taken at zero, 30, 60 and 90 seconds. The assumed final value for the elapsed period was the average of the 4 values.

2.5.6 Event related potentials – Auditory P300

ERP were evaluated in a silent room, using the 8-channel Keypoint.net v.2.03 device. The participants had to stay calm and focused on a certain reference point. Volunteers were instructed to press the button with their right hand as soon as they hear the target (oddball) stimulus.

After cleaning the scalp with alcohol, electrodes were placed at Fz, Cz and Pz locations of the 10/20 system, using a mastoid reference. As for processing, a time constant of 1 second, a high-frequency filter of 50 Hz, a time base of 1000 ms and an automatic rejection mode were applied. By a binaural stimulus, the oddball protocol was based on presenting 80% of a frequent stimulus, at 1000 Hz and 50 ms, and 20% of a randomly interspersed stimuli, at 2000 Hz and 100 ms, both having a minimal intensity of 70 dB. Each study recorded at least 80 target stimuli, in two reproducible series.

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The electrode application sites were cleaned with alcohol to maintain a resistance of less than 5 k Ω .

The largest negative peak, occurring between 160-260 ms, was considered N200. Prior to N200, the most prominent negative peak was marked – N100. P300 was defined as the largest positive peak occurring after the N1, P2 and N2 components, increasing in amplitude in the posterior areas and occurring between 220-600 ms. The amplitude was measured in the N2-P3 complex, between the maximum negative and positive components. To statistically analyzing the P300 peak, priority was given to assessing the Pz region, which is more clinically relevant. (26,27)

2.5.7 Inhibitory theta burst stimulation (continuous theta burst stimulation)

For the TBS protocol we used MagVenture MagPro® G3 x 100 5.0.1 magnetic stimulator, with an identical protocol of previous studies. Using Dantec TM Keypoint® electromyograph - Keypoint.net v.2.03, it was possible to obtain the stimulation point, by identifying the region that originated the greatest contraction of the contralateral abductor pollicis brevis muscle. (13)

TBS was applied to the left prefrontal cortex – 5 cm anterior to the previously defined hotspot on the left primary motor area. It was applied 80% of the activation intensity of the active motor threshold, as it is the minimum stimulation intensity for which there is a motor response in at least 5 of 10 stimuli, while the subject slightly contracts the contralateral abductor pollicis brevis muscle. (13) cTBS consisted in the application of TBS uninterruptedly, totaling 600 pulses. (25)

2.6 Statistical methods

The study data was analyzed using IBM SPSS Statistics v29.0.1 programs.

Data from quantitative variables were described by mean, standard deviations (sd), median, maximum (max), and minimum (min), while data from categorical variables were described by frequencies and percentages.

Friedman's test was used to analyze the effects of tests A and B, before and after TMS application. Kendall's W statistic was used for the effect size, the interpretation of which is similar to Cohen's interpretation (cut-off values of 0.1, 0.3 and 0.5 for small, moderate, and

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strong effects, respectively) (28). For pairwise comparisons, Dunn's test with Bonferroni correction was used. Correlations were measured using Spearman's correlation coefficient, since there are generally no linear trends.

Hypothesis tests whose proof value (p-value, p) did not exceed the significance level of 5% were considered significant.

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Chapter 3

Results

3.1 Sample description

Seven healthy young adult volunteers were studied. Table 2 gives the characteristics of volunteers' sample. It was predominantly constituted by females (n = 6), with a mean age of 24 years. Initially there were eight participants, but there was one dropout, because the individual commented to have had a disrupted sleep the night prior to test B.

Table 2 - Sample characterization.

| | |
|----------------------|--------------|
| Sex (n) (%) | |
| Female | 6 (85.71%) |
| Male | 1 (14.29%) |
| Age (years) – test A | |
| Mean ± sd | 24.00 ± 2.00 |
| Median (Min.; Max.) | 23 (22; 27) |
| Age (years) – test B | |
| Mean ± sd | 24.57 ± 2.32 |
| Median (Min.; Max.) | 23 (22; 28) |

At the time of the questionnaire (appendix II), volunteers were in the 5th (71.43%) and the 6th year (28.57%) of Medicine at the Faculty of Health Sciences of the University of Beira Interior.

Of the symptoms questioned in question 1 (appendix II) 57.13% (n=4) of the volunteers said they hadn't felt any, 14.29% (n=1) reported symptoms related to hypotension (more prevalent during summer) and 28.58% (n=2) to anxiety situations (monthly episodes). Only one volunteer (14.29%) stated that he suffered from some pathology – asthma (self-assessed as mild) and hypothyroidism, in questions 2 and 3 of appendix II.

Two volunteers (28.58%) stated that they had felt depressed before test A. Three (42.87%) volunteers expressed that they did not drink alcohol, and the other four (57.13%) only

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occasionally. Only one (14.19%) volunteer consumed tobacco, but less than 1 pack/day, and none did drugs.

Regarding physical exercise, 57.13% (n=4) of the volunteers practiced 0-60 minutes of aerobic exercise every week, and 42.87% (n=3) 0 minutes. Only one (14.29%) responded that performed strength exercise sessions – 1 session per week.

With respect to sleeping habits, 57.13% of volunteers slept near 7-8 hours a day, and one volunteer for each: 8-9 hours, 6-7 hours, and 5-6 hours.

3.2 Test A sample description

Relating to sleeping habits on the day of test A, table 3 presents the descriptive analysis of the number of hours slept the night before the test A, through mean, standard deviation, median, minimum, and maximum values. The median of hours slept was 6h and the median of relative number of hours slept was 80%.

Table 3 - Descriptive analysis of the number of hours slept the night before test A.

| Number of hours slept | | Relative number of hours slept ¹ (%) | |
|-----------------------|---------------------|---|---------------------|
| Mean ± sd | Median (min.; max.) | Mean ± sd | Median (min.; max.) |
| 5.78 ± 0.56 | 6 (5; 6.5) | 78.27±5.63 | 80 (67; 83) |

¹ The basal number of hours considered were the ones reported on the study day survey (appendix V)

The %HR_{res}, exercise was analyzed through mean, standard deviation, median, minimum, and maximum values. Table 4 shows that the minimal value was 40% and the maximal value was 53%, being the median 44%, classifying every subject's exercise as moderate.

Table 4 - Descriptive analysis of the mean %HRres achieved during exercise.

| %HR _{res} | |
|--------------------|---------------------|
| Mean ± sd | Median (min.; max.) |
| 45.71 ± 4.19 | 44 (40; 53) |

3.2 Test B sample description

Regarding sleeping habits on B test day, table 5 presents the descriptive analysis of the number of hours slept the night before test B, through mean, standard deviation, median, minimum, and maximum values. The median of hours slept was 6h and the median of relative number of hours slept was 88%.

Table 5 - Descriptive analysis of the number of hours slept the night before test B.

| Number of hours slept | | Relative number of hours slept ¹ (%) | |
|-----------------------|---------------------|---|---------------------|
| Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) |
| 6.76 \pm 1.03 | 6 (5.8; 8.25) | 90.86 \pm 11.01 | 88 (75; 107) |

¹ The basal number of hours considered were the ones reported on the study day survey (appendix V)

3.3 Chalder Scale (CS)

In table 6 is presented the descriptive analysis of the score obtained on the CS, through mean, standard deviation, median, minimum, and maximum values, on the test (A and B) day, 2 days, and 4 weeks follow-up.

In table 6 is also presented the inferential analysis, between CS punctuation (CSP) before TMS application and the 2 days follow-up, between the 2 days follow-up and the 4 weeks follow-up, and between CSP before TMS application and the 4 weeks follow-up.

On test A, the minimal punctuation obtained was 0 and the maximum 8. Giving 4 as the cut-off value was verified that the median is equal to this value. On test B, the minimal punctuation obtained was 0 and the maximum 5, and the median was 2.

Looking at the individuals' values, it can be said that the number of people considered fatigued using the CS (equal or more 4 points) were 4 on the day of test A, 1 on the 2 days follow-up of test A and again 4 on the 4 weeks follow-up of test A. On the day of test B, just 2 volunteers were fatigued, 1 on the 2 days follow-up, and 1 on the 4 weeks follow-up.

Comparing CSP before TMS application and the 2 days follow-up, the Friedman Test showed the existence of a significant effect ($p=0.009$). Kendall's W was 0.549, indicating a strong effect. However, the significant differences found result from comparisons that are

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not clinically relevant and as can be seen in table 7, the difference found while comparing clinical relatable moments were not significant.

Table 6 – Descriptive and inferential analysis of CS punctuation and pre and post stimulation.

| Variable (CSP) | Test A | | Test B | | p^1 (Kendall's W) |
|-------------------|-----------------|------------------------|-----------------|------------------------|------------------------------------|
| | Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) | |
| Test day | 4.00 \pm 2.30 | 4 (2; 8) | 2.14 \pm 1.86 | 2 (0; 5) | 0.009 (0.549) |
| 2 days follow-up | 2.00 \pm 2.00 | 2 (0; 6) | 1.71 \pm 2.56 | 1 (0; 7) | |
| 4 weeks follow-up | 3.71 \pm 3.35 | 3 (0; 8) | 1.86 \pm 1.77 | 2 (0; 5) | |
| Test day | x | x | x | x | (0.433) 0,136 (0,264) |
| 2 days follow-up | x | x | x | x | |
| 4 weeks follow-up | x | x | x | x | |

¹Friedman test

Table 7 – Comparison between CSP before test A and the 2 days follow-up CSP.

| Variable | Dunn test with Bonferroni correction | | | |
|----------|--------------------------------------|---------------------------|---------------------------------------|---------------------------------------|
| | Before A-B | After ¹ A-B | A (before and after ¹) | B (before and after ¹) |
| | p | p | p | P |
| CSP | 0,231 | 1 | 0,295 | 1 |

¹CS 2 days follow-up

On table 8, was compared CSP before the application of TMS with the 2-days follow-up and 4 weeks follow-up, it was found a significant correlation between CSP before test and the 2-day follow-up CSP ($p=0,012$; $rs=0,863$) and the 4 weeks follow-up ($p=0,014$; $rs=0,857$), and between the 2 days follow-up CSP and the 4 weeks follow-up ($p=0,034$; $rs=0,791$). It shows that test B (not altering lifestyle) did not disrupted significantly the perception of fatigue on the day of the test, differently of test A, showing that perception fatigue is somehow predicible on a daily base.

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Table 8 – Correlation between CSP before and after (2 days and 4 weeks follow-up) TMS application.

| | Variable | Test A | Test B | Test A | Test B | Test A | Test B |
|--------|-------------|----------------------|--------------------------------|----------------------|--------------------------------|----------------------|--------------------------------|
| | | CSP 2 days-follow-up | | CS 4 weeks follow-up | | CS 4 weeks follow-up | |
| Test A | CSP before | 0,397 (0,378) | x | x | x | x | x |
| Test B | TMS | x | 0,863 (0,012) | x | x | x | x |
| Test A | CSP before | x | x | 0,333 (0,465) | x | x | x |
| Test B | TMS | x | x | x | 0,857 (0,014) | x | x |
| Test A | CSP 2 days- | x | x | x | x | 0,619 (0,139) | x |
| Test B | follow-up | x | x | x | x | x | 0,791 (0,034) |

Values: rs (p-value)

3.4 Verbal fluency test

In table 9 is presented the descriptive analysis of verbal fluency test performance (VFP) using mean, standard deviation, median, minimum, and maximum values, as well as the inferential analysis, between the VFT before TMS application and VFT after it, and between the VFP before TMS application and the “new words” (NW) said after TMS application.

It was used the mean value of words said on M, P and R. The overall performance was better on B test day. Both median (17), minimum (15) and maximum (20) values are greater after the application of TMS. The Friedman Test showed the existence of a significant effect between VFP before and after TMS application ($p=0.008$). Kendall’s W was 0.569 indicating strong effect. However, the significant differences found result from comparisons that are not clinically relevant and as shown in table 10, the difference found while comparing clinical relatable moments were not significant.

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Table 9 – Descriptive and inferential analysis of VFP and pre and post stimulation.

| Variable (VFP) | Test A | | Test B | | p^1 (Kendall's W) |
|------------------------|------------------|---------------------|------------------|---------------------|--------------------------------|
| | Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) | |
| Before TMS | 12.76 \pm 2.46 | 12 (10.33; 18) | 15.57 \pm 1.75 | 15 (13; 18) | 0.008 (0.569) |
| After TMS | 14.38 \pm 3.10 | 15 (9.33; 19.67) | 17.43 \pm 1.89 | 17 (15; 20) | |
| New words ^a | 9.52 \pm 2.19 | 9 (7; 13.67) | 10.86 \pm 1.98 | 11 (8; 14) | x |

¹ Friedman test; ^a After TMS; ² Effect of test on VFT NW before TMS;

Table 10 – Comparison between VFP before TMS application and VFP after TMS application.

| Variable | Dunn test with Bonferroni correction | | | |
|------------------|--------------------------------------|------------------|-----------------------------|-----------------------------|
| | Before A-B p | After A-B p | A (before and after) P | B (before and after) p |
| VFP ¹ | 0,295 | 0,471 | 0,586 | 0,884 |

¹ Relation between VFP before and after TMS;

Despite literature indicating that fatigue and verbal fluency test performance are inversely related, table 11 shows that there was no significant correlation between the CSP and the performance on the verbal fluency test.

Table 11 – Correlation between CSP and VFP, before TMS.

| | Variable | Test A | Test B |
|--------|----------|---------------|---------------|
| | | CSP | |
| Test A | VFP | 0.406 (0.366) | x |
| Test B | | x | 0.066 (0.888) |

Values: r_s (p -value)

3.5 Near-Infrared Spectroscopy

In table 12 is presented the descriptive analysis of NIRS' results, through mean, standard deviation, median, minimum, and maximum values, on the test (A and B) day, as well as the inferential analysis, between the right hemisphere values on test A and test B, between

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the left hemisphere values on test A and test B, and between the right and left hemisphere values on test A and test B.

No significant difference was found in the median value on the oximetry of the right hemisphere and the left hemisphere between group A and group B, before TMS application. However, after TMS application we observed an increase of the median value on test A, but a decrease of the median value on test B, on both hemispheres. The Friedman Test however, did not show any significant difference.

Table 12 – Descriptive and inferential analysis of NIRS values and pre and post stimulation.

| Variable (NIRS) | Test A | | Test B | | p^1 (Kendall's W) | |
|-------------------------------|------------------|----------------------|------------------|----------------------|---------------------|---------------|
| | Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) | | |
| Right hemisphere ^a | 72.21 \pm 3.67 | 71.50 (68.50; 79.75) | 73.00 \pm 6.17 | 71.50 (66.25; 85.25) | 0.960 (0.010) | 0.540 (0.120) |
| Right hemisphere ^b | 72.86 \pm 5.45 | 71.75 (68.25; 84.25) | 71.89 \pm 8.04 | 68.25 (67.00; 84.50) | | |
| Left hemisphere ^a | 70.50 \pm 7.36 | 68.50 (62.50; 86.00) | 70.75 \pm 6.27 | 68.50 (63.50; 87.75) | 0.180 (0.20) | |
| Left hemisphere ^b | 72.11 \pm 7.19 | 69.50 (66.75; 87.75) | 72.00 \pm 8.53 | 68.00 (66.75; 90.50) | | |

¹ Friedman test; ^a Before TMS; ^b After TMS

As it is an easy tool to apply, it was looked for a correlation between CSP and NIRS' results. However as can be observed in table 13, no significant correlation was found.

Table 13 – Correlation between CSP before TMS and NIRS values.

| | Variable | Test A | | Test B | |
|--------|----------|----------------------------|----------------------------|----------------------------|-----------------------------|
| | | NIRS value before TMS | | | |
| Test A | CSP | 0,150 (0,749) ^a | 0,370 (0,937) ^b | x | x |
| Test B | | x | x | 0.190 (0.968) ^a | -0.425 (0.342) ^b |

Values: rs (p-value); ^a Right hemisphere; ^b Left hemisphere

3.6 Visual reaction test

In table 14 is presented the descriptive analysis of reaction time using mean, standard deviation, median, minimum, and maximum values, as well as the inferential analysis, between auditory reaction time (RT) before and after the application of TMS, on test A and test B.

It can be observed that the median time of RT decreased after TMS on both test A and test B, and that the median value is higher on test A, both before and after TMS application. However, the Friedman Test showed no significant effect ($p=0.940$).

Table 14 – Descriptive and inferential analysis of RT (milliseconds) and pre and post stimulation.

| Variable (RT) | Test A | | Test B | | p^1 (Kendall's W) |
|---------------|--------------------|---------------------|---------------------|---------------------|---------------------|
| | Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) | |
| Before TMS | 271.71 \pm 65.58 | 249 (200; 368) | 268.71 \pm 87.55 | 228 (201; 451) | 0.940 (0.020) |
| After TMS | 253.86 \pm 78.78 | 225 (205; 425) | 278.29 \pm 106.73 | 206 (202; 475) | |

¹ Friedman test;

There is a relation between hours of sleep and RT (29), that is somehow collaborated in table 15, as it was found a marginal inverse correlation between RT and the number of hours slept on test B ($p=0.058$; $r_s=-0.739$).

Table 15 – Correlation between RT and number of hours slept.

| | | Variable | Test A | Test B |
|--------|----|-----------------------|----------------|----------------|
| | | Number of hours slept | | |
| Test A | RT | | -0.214 (0.645) | x |
| Test B | | | x | -0.739 (0.058) |

Values: r_s (p-value)

3.7 Evoked potentials

In table 16 is presented the descriptive analysis of auditory evoked potentials using mean, standard deviation, median, minimum, and maximum values, as well as the inferential analysis, between EP before and after the application of TMS, on test A and test B.

In general, the absolute latency of P300 and N200 was superior on test A (slower evoked potentials), both before and after TMS application. Regarding amplitude, the absolute values were superior in test B, both before and after TMS application. In addition, the Friedman Test showed a significant effect between P300 before and after TMS application ($p=0.027$). Kendall's W was 0.437 indicating a moderate effect.

Table 17 gives the difference between latency of P300-Pz before and after TMS application. It was found a marginally significant difference ($p=0.078$) between the latency on test A, before and after TMS application, which seems to translate a difference in response when volunteers are fatigued (A) vs. non-fatigued (B).

Table 16 – Descriptive and inferential analysis of ERP and pre and post stimulation.

| Variable | Test A | | Test B | | p^1 (Kendall's W) |
|--------------------------------------|--------------------|------------------------|--------------------|------------------------|------------------------------|
| | Mean \pm sd | Median (min.; max.) | Mean \pm sd | Median (min.; max.) | |
| Latency of N100 - Fz ^a | 86.71 \pm 19.54 | 84 (56; 120) | 89.93 \pm 18.02 | 84 (68; 118) | 0.500 (0.112) |
| Latency of N100 - Fz ^b | 90.93 \pm 20.54 | 86 (71; 134) | 87.04 \pm 9.34 | 91 (76; 99) | |
| Latency of N100 - Cz ^a | 82.81 \pm 12.87 | 84 (61; 99) | 87.37 \pm 12.68 | 86 (71; 109) | 0.490 (0.116) |
| Latency of N100 - Cz ^b | 83.90 \pm 9.414 | 84 (71; 96) | 85.43 \pm 7.95 | 84 (73; 94) | |
| Latency of N100 - Pz ^a | 78.13 \pm 7.22 | 79 (68; 88) | 78.00 \pm 11.14 | 76 (68; 101) | 0.790 (0.048) |
| Latency of N100 - Pz ^b | 86.71 \pm 19.54 | 84 (56; 120) | 78.91 \pm 8.45 | 81 (66; 89) | |
| Latency of N200 - Fz ^a | 222.29 \pm 43.98 | 228 (142; 281) | 220.71 \pm 25.78 | 208 (187; 248) | 0.125 (0.273) |
| Latency of N200 - Fz ^b | 235.86 \pm 34.97 | 243 (167; 277) | 234.71 \pm 22.16 | 246 (192; 253) | |
| Latency of N200 - Cz ^a | 203.71 \pm 37.13 | 195 (144; 253) | 209.43 \pm 32.65 | 192 (177; 256) | 0.257 (0.193) |

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| | | | | | |
|-----------------------------------|----------------|----------------|-----------------|----------------|----------------|
| Latency of N200 - Cz ^b | 216.57 ± 34.86 | 210 (165; 258) | 223.57 ± 41.21 | 208 (177; 281) | |
| Latency of N200 - Pz ^a | 205.00 ± 36.75 | 200 (144; 258) | 210.00 ± 35.98 | 190 (175; 263) | 0.500 |
| Latency of N200 - Pz ^b | 215.00 ± 38.48 | 205 (165; 258) | 218.57 ± 45.617 | 195 (167; 286) | (0.112) |
| Latency of P300 - Fz ^a | 318.43 ± 24.32 | 316 (290; 368) | 327.57 ± 27.22 | 323 (304; 385) | 0.257 |
| Latency of P300 - Fz ^b | 337.43 ± 32.54 | 324 (304; 385) | 340.29 ± 48.96 | 319 (308; 448) | (0.193) |
| Latency of P300 - Cz ^a | 320.71 ± 13.77 | 319 (301; 433) | 331.43 ± 24.15 | 322 (304; 372) | 0.450 |
| Latency of P300 - Cz ^b | 343.29 ± 29.51 | 353 (309; 387) | 340.14 ± 39.16 | 334 (311; 423) | (0.126) |
| Latency of P300 - Pz ^a | 324.86 ± 14.44 | 323 (306; 349) | 330.71 ± 21.22 | 329 (306; 359) | 0.027 |
| Latency of P300 - Pz ^b | 350.71 ± 27.86 | 367 (314; 380) | 345.00 ± 38.05 | 339 (306; 423) | (0.437) |
| Amplitude P300 - Fz ^a | 15.27 ± 14.47 | 8 (7; 47) | 13.60 ± 4.37 | 12 (9; 19) | 0.930 |
| Amplitude P300 - Fz ^b | 15.69 ± 14.71 | 12 (4; 48) | 16.14 ± 38.05 | 14 (6; 35) | (0.020) |
| Amplitude P300 - Cz ^a | 14.54 ± 7.83 | 14 (5; 25) | 14.87 ± 7.39 | 15 (5; 25) | 0.930 |
| Amplitude P300 - Cz ^b | 14.27 ± 8.25 | 13 (3; 30) | 14.89 ± 7.34 | 17 (2; 25) | (0.020) |
| Amplitude P300 - Pz ^a | 13.46 ± 7.90 | 14 (4; 23) | 13.49 ± 6.49 | 16 (4; 24) | 0.860 |
| Amplitude P300 - Pz ^b | 13.09 ± 7.10 | 12 (3; 25) | 13.31 ± 6.99 | 15 (2; 24) | (0.037) |

¹ Friedman test; ^a Before TMS; ^b After TMS; Latency is in milliseconds; amplitude in microvolts (µV).

Table 17 – Comparison between latency of P300-Pz before and after TMS application (Dunn’ test with Bonferroni correction).

| Variable | Dunn test with Bonferroni correction | | | |
|-----------|--------------------------------------|----------|------------|------------|
| | Before | After A- | A (before | B (before |
| | A-B | B | and after) | and after) |
| | <i>p</i> | <i>p</i> | <i>p</i> | <i>p</i> |
| P300 - Pz | 1.000 | 1.000 | 0.078 | 0.884 |

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As example, the next figure (figure 1) shows the evoked potentials of one volunteer before and after cTBS stimulation.

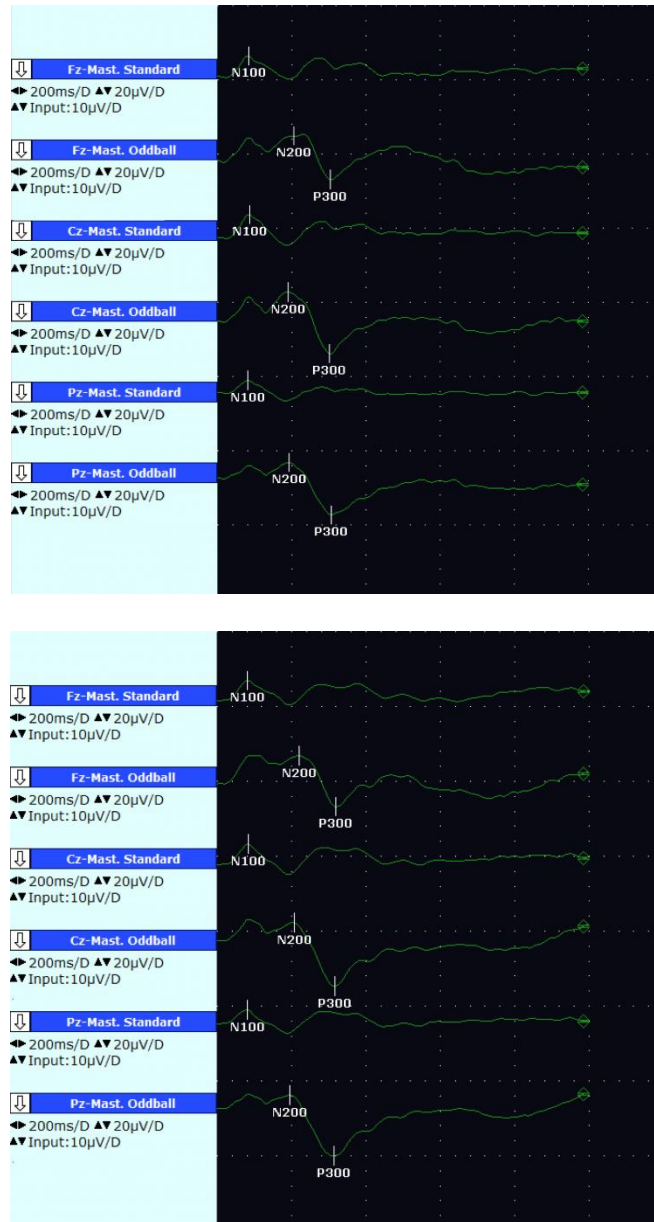


Figure 1 - Evoked potentials of the same volunteer before (1st picture) and after (2nd picture) cTBS stimulation.

In table 18 and table 19 is showed the correlation between CSP and ERP before TMS application.

Table 18 – Correlation between CSP and ERP, before TMS application, on test A.

| Variable | CSP |
|----------------------|----------------|
| Latency of N100 - Fz | -0,750 (0,873) |
| Latency of N100 - Cz | -0,374 (0,408) |

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| | |
|----------------------|----------------|
| Latency of N100 - Pz | -0,699 (0,080) |
| Latency of N200 - Fz | 0,262 (0,570) |
| Latency of N200 - Cz | -0,206 (0,658) |
| Latency of N200 - Pz | -0,206 (0,658) |
| Latency of P300 - Fz | 0.262 (0.570) |
| Latency of P300 - Cz | 0.168 (0.718) |
| Latency of P300 - Pz | 0.500 (0.253) |
| Amplitude P300 – Fz | -0,283 (0,538) |
| Amplitude P300 – Cz | -0,187 (0,688) |
| Amplitude P300 - Pz | -0,037 (0,937) |

Values: rs (p-value)

Table 19 – Correlation between CSP and ERP, before TMS application, on test B.

| Variable | CSP |
|----------------------|----------------|
| Latency of N100 - Fz | -0,393 (0,383) |
| Latency of N100 - Cz | -0,487 (0,268) |
| Latency of N100 - Pz | 0,028 (0,952) |
| Latency of N200 - Fz | 0,17 (0,716) |
| Latency of N200 - Cz | -0,112 (0,811) |
| Latency of N200 - Pz | -0,112 (0,811) |
| Latency of P300 - Fz | 0,112 (0,811) |
| Latency of P300 - Cz | -0,397 (0,379) |
| Latency of P300 - Pz | -0,225 (0,628) |
| Amplitude P300 – Fz | -0,299 (0,514) |
| Amplitude P300 – Cz | 0,019 (0,968) |
| Amplitude P300 - Pz | 0,160 (0,731) |

Values: rs (p-value)

It was not found any significant correlation between the number of hours slept and ERP latency and / or amplitude before TMS application, on test B, as shown in table 20.

Table 20 – Correlation between ERP and number of hours slept on test B.

| Variable | Number of hours slept |
|----------------------|-----------------------|
| Latency of N100 - Fz | -0.090 (0.848) |
| Latency of N100 - Cz | -0.036 (0.939) |
| Latency of N100 - Pz | -0.327 (0.474) |

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| | |
|----------------------|----------------|
| Latency of N200 - Fz | -0.136 (0.771) |
| Latency of N200 - Cz | -0.378 (0.403) |
| Latency of N200 - Pz | -0.378 (0.403) |
| Latency of P300 - Fz | 0.189 (0.670) |
| Latency of P300 - Cz | 0.564 (0.188) |
| Latency of P300 - Pz | 0.306 (0.504) |
| Amplitude P300 - Fz | 0.288 (0.531) |
| Amplitude P300 - Cz | 0.505 (0.248) |
| Amplitude P300 - Pz | 0.291 (0.527) |

Values: rs (p-value)

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Chapter 4

Discussion

The present study had three main objectives – to study the induced fatigue in healthy students, to evaluate the different effects of cTBS in fatigued and non-fatigued students and to understand which tools may be the most trustworthy to study fatigue. The subjects' perceived tiredness (mental and physical) was evaluated using CS. To assess subjects' cognitive state, it was used different quantitative parameters, such as verbal fluency test performance, cerebral oximetry, visual and auditive reaction time and cognitive evoked potentials.

Summarizing, we found that individuals felt more fatigued in test A and the disruption of lifestyle changed the perception of fatigue on long term. We also found a significant difference in the response of P300 latency after the application of cTBS in both tests, apparently more relevant in fatigued vs non-fatigued volunteers.

4.1 Chalder Scale

Chalder Scale was the tool used to evaluate the volunteers' perception of fatigue. Despite the variability of activities and life stressors of which students are exposed to, it was chosen to apply CS on the day of the study, as a 2-day follow-up and as a 4-week follow-up, as in previous works investigating the effects of TMS.

Considering the cut-off value of 4 (23), it was found that preceding test A the median value is 4, so volunteers can be considered fatigued – this is a meaningful result as the scores of the test B were almost half. Looking at short term effects (2-day follow-up) it can be observed a reduction of the perceived fatigue, a reduction that was not maintained. In test B, the median value is 2, so volunteers cannot be considered fatigued. There was also observed a reduction in the 2-day follow-up (1).

We found no significant difference between CSP before TMS application, on test A and on test B, however there is a difference of 50% in the median between test A and B. It is important to note that the median percentage of sleep on teste B was just 88%, reducing the

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comparison effect of test A and test B. Apart from that, there is a considerable variation of quotidian, adding another undesirable variable.

It was found a significant correlation between CSP before TMS application on test B and the 2 days follow-up ($p=0,012$; $rs=0,863$), between CSP before TMS application on test B and the 4 weeks follow-up ($p=0,014$; $rs=0,857$), and between the 2 days CSP follow-up on test B and the 4 weeks follow-up ($p=0,034$; $rs=0,791$) which indicates that the non-alteration of quotidian made CSP somehow predictable (26)(27).

The reduction of the perceived fatigue on the 2 days follow-up was 50% on both test A and on test B.

Overall, the population at which the study was applied may not be the best, because of the irregularity in the daily lives of UBI medical students – the scale was applied after holidays or exams, weeks with more or any classes. This counteracts the short-term analysis, because it was almost always applied in the weekend, making it more consistent and significant.

4.2 Verbal fluency test

Mental fatigue can be seen as cognitive impairment. (30) Verbal fluency test can be used to evaluate mental fatigue (10), so the objective of the study was to look at which degree it could be said that there is a relation between fatigue and performance on the verbal fluency test in test A and test B. As said before, it is known that there is an inverse relation between fatigue and VFP.

The overall performance was better on day B, indicating that some sleepiness associated with physical effort might induce a certain sort of fatigue, as expected when developing our protocol. However, there was not a major difference between VFP before test A and test B, making the effect of fatigue unclear, as said before.

It is also important to reinforce that the fatigue protocol designed for this study (test A) sought to evoke mild, not extreme, fatigue to guarantee the safety of the volunteers.

4.3 Near-Infrared Spectroscopy

At the beginning of a driving task there is an increase on the cerebral oxygenation, but the inference relation is seen in the end of it. (31) The realization of a moderate-intensity aerobic exercise is expected to increase both HbO₂ and HHb values (peak value in 20 minutes after beginning of exercise), on both sexes, but the difference between sexes is just significant in HHb's values. (32)

In our sample, there was no correlation between NIRS results and CSP results, which attest that it might not be a useful tool for studying perceived fatigue.

Apart from the reduced sample, it was not considered the volunteers' sex while studying the values of NIRS (also due to sample characteristics). (32)

4.4 Visual reaction test

Visual reaction time is intrinsic related with the central nervous system's processing capacity. (33) As said before, after a period of exercise there is a decrease of reaction time (24), but after a period of mental fatigue, as result of parasympathetic activity, there is an increase of reaction time. (33) Curiously, an increase in hours of sleep has a negative impact in RT; there seems to be a negative impact in RT when sleeping less hours. (29)

Despite male medical students have faster reaction times than female colleagues (34), the present study did not considered sex differences, due to the characteristics of the sample.

In this study was observed a higher mean on test A before TMS (271.71 ms) than the one in the validation study (269.050 ms) which had 35 hours of sleep deprivation. (25) But, the same was not verified on test B before TMS (268.71 ms). Considering that in test A were introduced two variables that point in different directions (exercise and sleep deprivation), the effect of sleepiness might be more important than what was observed.

As expected, it was found a marginal negative correlation between RT and the number of hours slept on test B ($p=0.058$; $r_s=-0.739$). The augment of the reaction time also observed with an increase of hours of sleep might indicate that there exist an optimal range of hours of sleep, in terms of visual reaction time.

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On both test A and test B it was observed an increase of the median value of RT, after cTBS application. The variability in the mean value is congruent with what was found on previous studies. (18)

4.5 Cognitive evoked potentials

Cognitive evoked potential N100 is intrinsically related with the primary auditive cortex. N200 and P300 are associated with maintenance of attention among other things. (35) Prolonged latency of both N200 and P300 were associated with moderate and severely fatigued individuals (36).

Acute physical exercise is somehow capable of reducing ERP's latency and augmenting P300 amplitude, but those finds are limited and is difficult to draw definitive conclusions (37) and in the present study to objectify any specific effect.

After application of cTBS it was observed an increase on the latency of N200 and P300, on both test A and test B. In a previous study of Pinto *et al.* (2018) it was also observed a delay of P300 after cTBS. (18) After application of cTBS, latency of P300 - Pz on test A was more prolonged on test B.

An increase of P300 latency seems to represent a cognitive processing struggle (38), possibly due to abnormal changes in acetylcholine and dopamine concentrations related to TBS. especially in the more fatigued brain. (17)

4.6 Follow-up and adverse effects

There was not registered any side effect of cTBS, neither during sessions, 2-days follow-up nor 4-weeks follow-up, which indicates that the protocol applied can be used in future studies.

4.7 Study limitations

Considering the sample we studied, there are 2 major problems – it was very reduced (n=7) and female individuals were clearly predominant (n=6).

Carrying out TMS required no coffee to be ingested 12 hours before the sessions, which accounting that test B would be unchanged, an important stimulant was removed and therefore there was some induction of fatigue, which is not being taken into consideration – so daily coffee intake should have been analyzed and a limit established according to the possibility of addiction. Even though TMS was applied in 2 sessions it was not clear the effect it had on the fatigue, and vice versa, because of the irregularity on the volunteers' daily lives.

It was taken in consideration the number of hours the volunteers slept the night before, but not the number of hours the volunteers were awoken.

Furthermore, it would have been important to measure the recovery heart rate, trying to understand to which extent the cardiovascular response adjusts to the aging of the cardiovascular system, and therefore to fatigue.

4.8 Future considerations

For future studies it would be important to apply this methodology to a population with a more stable quotidian, as it could make it easier to analyze with higher reliability the effects of inducing fatigue.

Another changing feature of the study would be the application of a depression evaluation scale – as it could be used to explore the effects of depression on certain biological parameters as well as taking in consideration fatigue as an important differential diagnosis of depression. It would have been important to make a follow-up of fatigue one week before the test to see if the sleep fluctuation is important to the perceived fatigue. Apart from that it would be important to use/ create a scale that allowed to study tiredness as a spectrum (not just fatigued vs non-fatigued individuals), making it more reliable for research studies.

As considered very common in the general population and having an important impact on the study of fatigue, for future research could be crucial to apply a scale of anxiety and depression, as it could make clearer the effect of those two entities.

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Despite being the most used letters in the phonemic verbal fluency test in the Portuguese language, those may not be the most convenient ones, as some authors defend that Q, T and P are the ones that may possibly be the best discriminate between the existence or not of cognitive decline (10). It would be also interesting to study the importance of applying the methodology of using the “new words” for seeking fatigue induced activities.

Looking to NIRS, it should be used as a tool for measuring momentaneous fatigue in future studies, so while provoking cognitive fatigue the variation of HBB and HbO₂ could be measured, and we could analyze the extent to which autoregulation is, or a limiting factor to study fatigue, or a fatigue cut-off value. This same methodology could have been applied to verbal fluency test and visual reaction test.

Looking at the visual reaction test, volunteers commented that in the second time doing it they felt as having better results because they had already experienced it. So, for future studies it might be important to consider the values given by a second encounter, or to study at which extent this second time improvement might be true/ significant. In terms of protocol, physical exercise and sleep deprivation counteract, so inducing cognitive and physical fatigue might have reduced the importance of RT as a tool to measure prolonged fatigue.

If used s2P to the evaluation of sleepiness, it would be important to study the number of false starts, as they are more common in sleepy-state conditions. (25) Also, because of the difficulty to evaluate volunteers at the exact same condition, it would be important to evaluate RT during a regular day.

Chapter 5

Conclusion

The present study intended to evaluate the effects of inducing fatigue with cTBS application and evaluate quantitatively the fatigue induced, in healthy volunteers.

We observed that verbal fluency test performance, both overall and the number of new words, may be used to look for tiredness. Reaction visual time may also be negatively affected by fatigue. It was also concluded that ERPs might have important roles in studying cognitive impairment associated to fatigue. Due to limitations of our sample, results presented must be read with caution and studies with more volunteers are clearly needed. Future investigations on biological markers of fatigue are incredibly important for the application of them in subsequent guidelines or studies protocols.

In conclusion, the use of cTBS might have a stronger effect on fatigued individuals vs non-fatigued ones. Application of cTBS on different populations (healthy and not healthy) and different professions, in a bigger scale, may be important to understand the utility of this protocol. Due to the absence of negative effects of this protocol, it should be used to further understand the mechanism behind fatigue among others, so that they can be prevented or treated more efficiently.

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Bibliography

1. Maisel P, Baum E, Donner-Banzhoff N. Fatigue as the Chief Complaint. *Dtsch Arztebl Int* [Internet]. 2021 Aug 23;118(33):566–76. Available from: <https://www.aerzteblatt.de/10.3238/arztebl.m2021.0192>
2. Abd-Elfattah HM, Abdelazeim FH, Elshennawy S. Physical and cognitive consequences of fatigue: A review. *J Adv Res* [Internet]. 2015 May 1;6(3):351–8. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2090123215000235>
3. McMorris T, Barwood M, Hale BJ, Dicks M, Corbett J. Cognitive fatigue effects on physical performance: A systematic review and meta-analysis. *Physiol Behav* [Internet]. 2018 May 1;188:103–7. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0031938418300441>
4. Goel N, Basner M, Rao H, Dinges DF. Circadian Rhythms, Sleep Deprivation, and Human Performance. In: *Progress in Molecular Biology and Translational Science* [Internet]. Elsevier B.V.; 2013. p. 155–90. Available from: <https://linkinghub.elsevier.com/retrieve/pii/B9780123969712000075>
5. Jackson CA, Earl L. Prevalence of fatigue among commercial pilots. *Occup Med (Chic Ill)* [Internet]. 2006 Jun 1;56(4):263–8. Available from: <http://academic.oup.com/occmed/article/56/4/263/1540971/Prevalence-of-fatigue-among-commercial-pilots>
6. Buczaj A, Pecyna A, Krzywicka M, Maruszewska A. Phenomenon of fatigue among professional drivers in Poland. *Annals of Agricultural and Environmental Medicine* [Internet]. 2020 May 27;28(1):114–21. Available from: <http://www.journalssystem.com/aaem/Phenomenon-of-fatigue-among-professional-drivers-in-Poland,122302,0,2.html>
7. Mollica RF, Fricchione GL. Mental and physical exhaustion of health-care practitioners. Vol. 398, *The Lancet*. Elsevier B.V.; 2021. p. 2243–4.
8. Cordeiro AP. Adaptação portuguesa do questionário checklist of individual strength (cis20-p): análise das propriedades psicométricas [Internet]. Lisboa; 2012 [cited 2022 Jul 31]. Available from: <https://repositorio.ispa.pt/bitstream/10400.12/3781/1/14979.pdf>

Exhausted

9. Tanaka M, Tajima S, Mizuno K, Ishii A, Konishi Y, Miike T, et al. Frontier studies on fatigue, autonomic nerve dysfunction, and sleep-rhythm disorder. *The Journal of Physiological Sciences* [Internet]. 2015 Nov 29;65(6):483–98. Available from: <http://link.springer.com/10.1007/s12576-015-0399-y>
10. Pereira Braz V. Quais as Melhores Letras na Língua Portuguesa Para Testar a Fluência Verbal Fonémica e Discriminar a Existência e Ausência de Déficit Cognitivo? Estudo Preliminar. Coimbra; 2017 Mar.
11. Wu P, Wang C, Wei M, Li Y, Xue Y, Li X, et al. Prefrontal cortex functional connectivity changes during verbal fluency test in adults with short-term insomnia disorder: a functional near-infrared spectroscopy study. *Front Neurosci*. 2023;17.
12. O’Shea DM, De Wit L, Szymkowicz SM, McLaren ME, Talty F, Dotson VM. Anxiety Modifies the Association between Fatigue and Verbal Fluency in Cognitively Normal Adults. *Archives of Clinical Neuropsychology* [Internet]. 2016 Sep 6; Available from: <https://academic.oup.com/acn/article-lookup/doi/10.1093/arclin/acw045>
13. Pinto N, Gonçalves H, Silva R, Duarte M, Gama J, Vaz Pato M. Theta burst stimulation over the prefrontal cortex: Effects on cerebral oximetry and cardiovascular measures in healthy humans. *Neurosci Lett* [Internet]. 2021 May 1 [cited 2023 Sep 27];752:135792. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0304394021001701>
14. Jung J, Lambon Ralph MA. The immediate impact of transcranial magnetic stimulation on brain structure: Short-term neuroplasticity following one session of cTBS. *Neuroimage* [Internet]. 2021 Oct;240:118375. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1053811921006510>
15. van Dinteren R, Arns M, Jongsma MLA, Kessels RPC. P300 Development across the Lifespan: A Systematic Review and Meta-Analysis. Di Russo F, editor. *PLoS One* [Internet]. 2014 Feb 13 [cited 2022 Aug 9];9(2):e87347. Available from: <https://dx.plos.org/10.1371/journal.pone.0087347>
16. Krigolson OE, Hammerstrom MR, Abimbola W, Trska R, Wright BW, Hecker KG, et al. Using Muse: Rapid Mobile Assessment of Brain Performance. *Front Neurosci* [Internet]. 2021 Jan 28 [cited 2022 Aug 9];15. Available from: <https://www.frontiersin.org/articles/10.3389/fnins.2021.634147/full>
17. Frodl-Bauch T, Bottlender R, Hegerl U. Neurochemical substrates and neuroanatomical generators of the event-related P300. *Neuropsychobiology*

Exhausted

- [Internet]. 1999 [cited 2024 Jan 2];40(2):86–94. Available from: <https://pubmed.ncbi.nlm.nih.gov/10474063/>
18. Pinto N, Duarte M, Gonçalves H, Silva R, Gama J, Pato MV. Bilateral theta-burst magnetic stimulation influence on event-related brain potentials. Yuan T, editor. *PLoS One* [Internet]. 2018 Jan 5;13(1):e0190693. Available from: <https://dx.plos.org/10.1371/journal.pone.0190693>
 19. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, et al. National Sleep Foundation’s sleep time duration recommendations: methodology and results summary. *Sleep Health* [Internet]. 2015 Mar 1;1(1):40–3. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2352721815000157>
 20. WHO guidelines on physical activity and sedentary behaviour.
 21. Nikolaidis P. Age-predicted vs. measured maximal heart rate in young team sport athletes. *Nigerian Medical Journal* [Internet]. 2014;55(4):314. Available from: <http://www.nigeriamedj.com/text.asp?2014/55/4/314/137192>
 22. HHS. *Physical Activity Guidelines for Americans 2nd edition*. Washington, DC; 2018.
 23. José da Silva F. *A capacidade para trabalho e a fadiga entre trabalhadores de enfermagem*. São Paulo; 2011.
 24. Pavelka R, Třebický V, Třebická Fialová J, Zdobinský A, Coufalová K, Havlíček J, et al. Acute fatigue affects reaction times and reaction consistency in Mixed Martial Arts fighters. Liao YH, editor. *PLoS One* [Internet]. 2020 Jan 31 [cited 2022 Aug 9];15(1):e0227675. Available from: <https://dx.plos.org/10.1371/journal.pone.0227675>
 25. Brunet JF, Dagenais D, Therrien M, Gartenberg D, Forest G. Validation of sleep-2-Peak: A smartphone application that can detect fatigue-related changes in reaction times during sleep deprivation. *Behav Res Methods* [Internet]. 2017 Aug 8;49(4):1460–9. Available from: <http://link.springer.com/10.3758/s13428-016-0802-5>
 26. Schomer D. *Niedermeyer’s Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*. 7th ed. Oxford University Press; 2017.
 27. Tsolaki A, Kosmidou V, Hadjileontiadis L, Kompatsiaris I, Tsolaki M. Brain source localization of MMN, P300 and N400: aging and gender differences. *Brain Res* [Internet]. 2015 Apr 7 [cited 2024 Feb 26];1603:32–49. Available from: <https://pubmed.ncbi.nlm.nih.gov/25445998/>

Exhausted

28. Marshall E, Marquier Reviewer B, Knox C. Friedman test in SPSS (Non-parametric equivalent to repeated measures ANOVA) [Internet]. Amsterdam; 2020. Available from: www.statstutor.ac.uk
29. Henry A, Katsoulis M, Masi S, Fatemifar G, Denaxas S, Acosta D, et al. The relationship between sleep duration, cognition and dementia: a Mendelian randomization study. *Int J Epidemiol* [Internet]. 2019 Jun 1 [cited 2023 Oct 31];48(3):849–60. Available from: <https://academic.oup.com/ije/article/48/3/849/5486060>
30. Campos MC, Nery T, Starke AC, de Bem Alves AC, Speck AE, S Aguiar A. Post-viral fatigue in COVID-19: A review of symptom assessment methods, mental, cognitive, and physical impairment. *Neurosci Biobehav Rev* [Internet]. 2022 Nov 1 [cited 2023 Oct 29];142:104902. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0149763422003918>
31. Li Z, Zhang M, Zhang X, Dai S, Yu X, Wang Y. Assessment of cerebral oxygenation during prolonged simulated driving using near infrared spectroscopy: its implications for fatigue development. *Eur J Appl Physiol* [Internet]. 2009 Oct 4;107(3):281–7. Available from: <http://link.springer.com/10.1007/s00421-009-1122-6>
32. Inagaki Y, Sato R, Uchiyama T, Kojima S, Morishita S, Qin W, et al. Sex differences in the oxygenation of the left and right prefrontal cortex during moderate-intensity exercise. *Int J Environ Res Public Health*. 2021 May 2;18(10).
33. Tchounwou B, Kreider RB, Mario Migliaccio G, Di Filippo G, Russo L, Orgiana T, et al. Effects of Mental Fatigue on Reaction Time in Sportsmen. *Res Public Health* [Internet]. 2022 [cited 2023 Oct 31];19:14360. Available from: <https://doi.org/10.3390/ijerph192114360>
34. Jain A, Bansal R, Kumar A, Singh K. A comparative study of visual and auditory reaction times on the basis of gender and physical activity levels of medical first year students. *Int J Appl Basic Med Res* [Internet]. 2015 [cited 2023 Oct 31];5(2):124. Available from: <https://journals.lww.com/10.4103/2229-516X.157168>
35. Kaga K, Fukami T, Masubuchi N, Ishikawa B. Effects of button pressing and mental counting on N100, N200, and P300 of auditory-event-related potential recording. *Journal of International Advanced Otology*. 2014;10(1):14–8.
36. Pokryszko-Dragan A, Zagrajek M, Slotwinski K, Bilinska M, Gruszka E, Podemski R. Event-related potentials and cognitive performance in multiple sclerosis patients

Exhausted

- with fatigue. *Neurological Sciences* [Internet]. 2016 Sep 1 [cited 2023 Dec 28];37(9):1545. Available from: /pmc/articles/PMC4992503/
37. Gusatovic J, Gramkow MH, Hasselbalch SG, Frederiksen KS. Effects of aerobic exercise on event-related potentials related to cognitive performance: a systematic review. *PeerJ*. 2022 Jul 11;10.
 38. Jia H, Joe Lin C, Min-yang Wang E. Effects of mental fatigue on risk preference and feedback processing in risk decision-making. *Scientific Reports* | [Internet]. 123AD [cited 2024 Feb 17];12:10695. Available from: <https://doi.org/10.1038/s41598-022-14682-0>

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Appendices

I – Consentimento Informado

Antonio Alejandro Villasana De Abreu, aluno do Mestrado Integrado em Medicina da Faculdade de Ciências da Saúde da Universidade da Beira Interior, a realizar um trabalho de investigação no âmbito da Tese de Mestrado, subordinada à tese “Exhausted” vem solicitar a sua colaboração neste estudo.

O estudo incide sobre uma amostra de voluntários com idades entre os 18-30, inclusive, pertencentes à população de alunos da Faculdade de Ciências da Saúde da Universidade da Beira Interior, que não receberão nenhum benefício pela participação no estudo. São critérios de exclusão para o mesmo:

- Indivíduos portadores de elementos metálicos na região do crânio (excluindo região oral);
- Antecedentes pessoais de convulsões ou crises epiléticas;
- Diagnóstico de patologia neurológica, cardíaca, respiratória ou psiquiátrica major;
- Patologia que provoque aumento da pressão intracraniana;
- Cirurgia neurológica prévia;
- Confirmação ou suspeita de gravidez;
- Indivíduos sob medicação que reduza o limiar convulsivante, sem toma concomitante de anticonvulsivante;
- Alcoolismo ou uso de drogas ilícitas;
- Dormir menos de 6 horas ou mais de 11 horas diárias;
- Realização de mais de 300 minutos de exercício aeróbio e mais de 3 sessões de exercício de força, por semana.

De forma resumida, o estudo consiste na divisão dos voluntários em 2 grupos de sujeitos que serão expostos a estimulação magnética transcraniana ou a estimulação placebo. Previamente, serão procedidos a exames na invasivos de medição de fadiga. Após a estimulação, estas serão novamente aplicadas.

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Ao todo, a intervenção terá um total de cerca de 90 minutos, por sessão – um total de 180 minutos nas duas sessões.

Este estudo mereceu parecer favorável da Comissão de Ética.

O/a voluntário/a foi informado/a acerca dos riscos e possíveis efeitos secundários associados à técnica aplicada, que são os seguintes, de acordo com o consenso de segurança para a EMT – Rossi et al, 2009:

- Ruído junto do ouvido durante a aplicação dos pulsos magnéticos que alguns sujeitos podem considerar desagradável
- Convulsões (raro - apenas 1 caso reportado desde 2005, num sujeito com privação de sono e estimulação com intensidade superior ao protocolado)
- Síncope (como epifenómeno, não relacionado com o efeito cerebral direto)
- Cefaleia transitória ocasional
- Desconforto local (escalpe, dentes ou pescoço) transitório
- Parestesias transitórias no hemicorpo contralateral e dependendo do local de estimulação

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Assinatura do responsável: _____

Eu, _____, nascido a ___/___/_____, natural de _____, residente em _____, distrito _____, e nacional de _____, declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pela pessoa que acima assina. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo/a investigador/a.

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Assinatura do voluntário: _____

Covilhã, __ / __ / ____

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II – Questionário aos Voluntários

As perguntas a seguir apresentadas serviram como base ao forms utilizado.

Código: _____

Sexo: Feminino Masculino

Profissão: _____

Escolaridade: _____

1. Alguma vez sofreu de algum destes sintomas (preencha em caso afirmativo)?

Síncope Disestesias

Palpitações Parestesias

Tonturas Alterações da visão

Cefaleias Alterações da fala

Convulsões ou outras crises epiléticas

Alterações da força

Acufenos/zumbidos/problemas auditivos

Outro(s) _____ - _____

Se preencheu algum parâmetro, por favor responda às seguintes questões, especificando qual dos sintomas a que se refere:

Em que circunstâncias ocorreu o episódio? _____

Quanto tempo decorreu desde a última sintomatologia? _____

Com que frequência (diário/semanal/mensal/ anual)? _____

Quanto tempo costumam durar os episódios? _____

Já consultou um médico, pelas condições acima referidas?

Sim Não

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Em caso de resposta afirmativa:

Qual o diagnóstico final? _____

2. Sofre de alguma patologia cardíaca, respiratória ou neurológica/psiquiátrica?

Sim Não

Em caso de resposta afirmativa:

Qual?

3. Já lhe foi diagnosticada algum outro tipo de doença, que não referida anteriormente?

Sim Não

Em caso de resposta afirmativa indique qual(ais):

4. Já teve algum episódio de traumatismo craniano?

Sim Não

Em caso de resposta afirmativa:

Quando? _____

Em que circunstância? _____

Existiram complicações após o traumatismo? _____

Em caso de resposta afirmativa:

O que lhe foi diagnosticado? _____

Continua a ser acompanhado(a) por algum médico? _____

5. Já alguma vez foi submetido a alguma cirurgia?

Sim Não

Em caso de resposta afirmativa:

Qual foi a causa? _____

Surgiu algum tipo de complicações durante o processo (pré, durante e pós cirurgia)? _____

Em caso de resposta afirmativa:

Qual (ais)? _____

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6. Faz, atualmente, alguma medicação?

Sim Não

Qual? _____ Causa: _____

Qual? _____ Causa: _____

Qual? _____ Causa: _____

7. Tem algum tipo de prótese metálica? (no cérebro, implantes cocleares, neuroestimulador, pacemaker ou linhas cardíacas, aparelho de infusão de medicação).

Sim Não

Em caso de resposta afirmativa:

Onde? _____

8. Tem alguma derivação ventricular ou medular?

Sim Não

9. Usa aparelho dentário?

Sim Não

10. Usa piercings?

Sim Não

Em caso de resposta afirmativa:

Onde? _____

11. Está grávida ou tem possibilidade de estar grávida?

Sim Não

12. Tem-se sentido deprimido(a), em baixo, sem motivação, nas últimas duas semanas?

Sim Não

13. Já fez RMN-CE ou medular alguma vez?

Sim Não

14. Já fez ETM alguma vez?

Sim Não

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15. Qual o seu membro dominante?

Direito

Esquerdo

Ambidextro

16. Quanto aos seus hábitos

16.1 Na última semana, quantas horas dormiu por noite?

>11h

10h a 11h

9h a 10h

8h a 9h

7h a 8h

6h a 7h

5h a 6h

4h a 5h

<4h

16.2 Na última semana, quantos dias saiu à noite?

0

1 ou 2

3 ou 4

5 ou 6

7

16.3 Consome álcool?

Sim Não

Em caso afirmativo responda aos dois pontos abaixo,

Diariamente

Semanalmente

Ocasionalmente

O que consome/consumiu e em que quantidades: _____

16.4 Consome drogas?

Sim Não

Em caso afirmativo responda aos dois pontos abaixo,

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Diariamente

Semanalmente

Ocasionalmente

O que consome/consumiu e em que quantidades: _____

16.5 Consome tabaco?

Sim Não

Em caso afirmativo, consome mais de um maço de tabaco por dia?

Sim Não

16.6 Quantos minutos de exercício aeróbico pratica por semana, em média?

0 minutos

0 a 60 minutos

60 a 120 minutos

120 a 180 minutos

180 a 240 minutos

240 a 300 minutos

>300 minutos

16.7 Quantos sessões de exercício de força pratica por semana, em média?

0

1

2

3

>3

16.8 Consume chá, café ou outras bebidas que contenham substâncias estimulantes (cafeína, teína, taurina, entre outras) e em que quantidades? Se for consumo esporádico, não considerar.

Exhausted

Assinatura do responsável: _____

Assinatura do voluntário: _____

Covilhã, ___ / ___ / _____

II.a – Questionário aos Voluntários

Nome: _____

Data de nascimento: ___/___/_____

Naturalidade: _____

BI/ cartão de cidadão: _____

Código: _____

A informação cedida no questionário de voluntários preenchido é para uso exclusivo do projeto e todas as respostas dadas serão mantidas em extrema confidencialidade e só serão ligadas ao nome da pessoa em casos de dúvida ou esclarecimento de alguns parâmetros.

Assinatura do responsável: _____

Assinatura do voluntário: _____

Covilhã, ___ / ___ / _____

Exhausted

III – Instruções preparatórias

Caro participante, de forma a garantir os resultados mais fidedignos na investigação em curso, pede-se que na véspera da realização dos testes sigas as seguintes instruções:

1. Não beber chá, café ou outras bebidas que contenham substâncias estimulantes (caféina, teína, taurina, entre outras) nas 12h prévias à realização dos exames
2. Não consumir álcool nas 24 horas anteriores aos exames
3. No dia do exame, lavar o cabelo e não aplicar laca, gel ou espuma no cabelo

Na véspera do teste A deverá ser procedida a uma redução dos hábitos noturnos em 20%, e na véspera do teste B não se deverá proceder a nenhuma alteração. Ainda se pede que para o dia do teste A traga roupa e calçado confortável para a realização da marcha.

Obrigado pela sua ajuda.

Os melhores cumprimentos,

A equipa de investigação

Exhausted

IV – Inquérito do dia de estudo

Código: _____

Na última noite, quantas horas dormiu?

- >11h
- 10h a 11h
- 9h a 10h
- 8h a 9h
- 7h a 8h
- 6h a 7h
- 5h a 6h
- 4h a 5h
- <4h

Explícite o número de horas: _____

Na última semana, quantas horas dormiu por noite?

- >11h
- 10h a 11h
- 9h a 10h
- 8h a 9h
- 7h a 8h
- 6h a 7h
- 5h a 6h
- 4h a 5h
- <4h

Explícite o número de horas: _____

Na última semana, quantos dias saiu à noite?

- 0
- 1 ou 2
- 3 ou 4
- 5 ou 6
- 7

Consumiu álcool nas últimas 24h? Sim Não

Exhausted

Consumiu chá, café ou outras bebidas que contenham substâncias estimulantes (cafeína, teína, taurina, entre outras) nas últimas 12 horas? Sim

Não

A qualidade do sono foi sobreponível à desta semana? Sim Não

Se não, explicita de que forma:

Exhausted

| Total | | | | | |
|----------------|----------------|----------------|----------------|----------------|----------------|
| M | | P | | R | |
| <i>Pré TMS</i> | <i>Pós-TMS</i> | <i>Pré TMS</i> | <i>Pós-TMS</i> | <i>Pré TMS</i> | <i>Pós TMS</i> |
| | | | | | |
| Novas pós: | | Novas pós: | | Novas pós: | |

Assinatura do responsável: _____

Covilhã, ___ / ___ / _____

Annexes

I – Parecer da comissão ética



UNIVERSIDADE DA BEIRA INTERIOR
FACULDADE DE CIÊNCIAS DA SAÚDE

PARECER

Processo: CE-FCS-2011-001

Data entrega processo: 30/08/2011

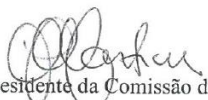
Data conclusão processo: 13/12/2011

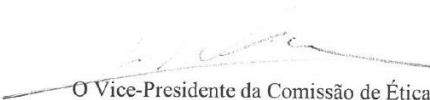
Tema Projecto/Proponente: *“Estudo das respostas neuropsicológicas e neurofisiológicas cerebrais, em sujeitos normais, associadas à aplicação de campos magnéticos cerebrais”* – Prof.^a Doutora Maria Assunção Vaz Patto

Exma. Sra. Presidente da Faculdade de Ciências da Saúde

Apreciado o pedido referente ao processo acima mencionado esta Comissão não detectou matéria que ofenda os princípios éticos.

Covilhã, 11 Janeiro 2012


O Presidente da Comissão de Ética
Prof. Doutor José Martinez de Oliveira


O Vice-Presidente da Comissão de Ética
Prof. Doutor Joaquim Viana

Exhausted

II – Escala de Chalder

Código: _____

Em relação às duas últimas semanas, por favor, marque com x as condições, de acordo com as opções ao lado.

| Fadiga Física | Nunca 0 | Raramente 1 | Às vezes 2 | Sempre 3 |
|---|------------|----------------|---------------|-------------|
| Eu cansei-me facilmente | | | | |
| Precisei de descansar mais | | | | |
| Estive sonolento | | | | |
| Não consegui começar nada | | | | |
| Estive com falta de ânimo | | | | |
| Senti menos força muscular | | | | |
| Senti-me fraco | | | | |
| Fadiga mental | | | | |
| Tive problemas de concentração | | | | |
| Tive dificuldade para pensar de forma clara | | | | |
| Tive dificuldade para encontrar a palavra certa | | | | |
| Tive problemas de memória | | | | |

Assinatura do responsável: _____

Covilhã, ___ / ___ / _____

