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**TRANSCRANIAL FOCUSED ULTRASOUND STIMULATION FOR TREATMENT-
REFRACTORY DEPRESSION**

Belo Horizonte

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REFRACTORY DEPRESSION**

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I hereby dedicate this thesis to all
nameless erudite researchers and
professors, so often undervalued, who
struggle daily to contribute to the ascent of
scientific enlightenment. Let their
veneration echoes throughout the
hallowed precincts of academia, and may
forthcoming generations be incited to
ponder profoundly upon the respect they
deserve.

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“The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter — for the future. His duty is to lay the foundation for those who are to come, and point the way.”

TESLA, Nikola.

RESUMO EM LÍNGUA VERNÁCULA

Introdução: O ultrassom focalizado transcraniano (tFUS) tem sido estudado na última década como uma ferramenta potencial para neuromodulação em humanos. Embora os estudos até o momento sejam promissores, a tradução para a prática clínica ainda é desafiadora.

Objetivos: O objetivo primário deste estudo é desenvolver uma metodologia reprodutível e clinicamente confiável para ultrassom focalizado transcraniano de baixa intensidade em pacientes com depressão resistente ao tratamento (TRD). Para enfrentar os desafios de implementação de um protocolo terapêutico, foram desenvolvidos uma revisão sistemática, bem como a otimização da neuronavegação e do direcionamento do alvo. Essas etapas foram necessárias para, por fim, desenvolver um protocolo de neuroestimulação e um estudo piloto.

Métodos: Cada etapa do processo é composta por seus próprios métodos. Os materiais evoluíram ao longo da tese para se adequar ao hardware e ao software disponíveis no Centro de Tecnologia Molecular da Universidade Federal de Minas Gerais.

Resultados: Foi realizada uma revisão sistemática que descreveu a evolução da pesquisa em tFUS em humanos ao longo dos anos. Não foi encontrado nenhum estudo em humanos para tratar depressão. A neuronavegação foi considerada uma preocupação importante, especialmente para alvos cerebrais profundos. Assim, um método de neuronavegação de código aberto foi otimizado para oferecer maior facilidade de uso e para ser adotado em um futuro ensaio clínico. Para aprimorar os procedimentos de direcionamento do alvo, foi estudado um modelo computacional do Sistema Internacional 10–20, a fim de posicionar corretamente o transdutor em tempo real durante a neuronavegação. Por fim, foi estabelecido um protocolo de ensaio clínico utilizando o software e o hardware disponíveis, culminando em um estudo piloto com o primeiro paciente incluído.

Conclusões: O tFUS é um método promissor de neuromodulação. Esta tese apresenta melhorias nos procedimentos de neuromodulação e propõe um ensaio clínico para avaliar a segurança e a eficácia de sessões repetidas para TRD. Contudo, ainda há necessidade de replicação desses métodos por diferentes centros, bem como de avaliação de segurança e eficácia.

Palavras-chave: ultrassom focalizado transcraniano; depressão refratária ao tratamento; neuromodulação; estimulação magnética transcraniana; neuronavegação.

RESUMO EM LÍNGUA ESTRANGEIRA

ABSTRACT

Introduction: The transcranial focused ultrasound (tFUS) has been studied over the past decade as a potential tool for neuromodulation in humans. Although studies have been promising so far, the translation into clinical practice is still challenging.

Objectives: The primary objective of this study is to develop a reproducible, clinically reliable methodology for low-intensity transcranial focused ultrasound in patients with treatment-resistant depression (TRD). To address the challenges to implement a treatment protocol, a systematic review, as well as optimization of the neuronavigation and targeting were developed. These steps were needed to finally develop a neurostimulation protocol and a pilot study.

Methods: Each step of the process is composed by its own methods. The materials evolved during the course of the thesis to fit into the hardware and software available in the Centro de Tecnologia Molecular at the Federal University of Minas Gerais.

Results: A systematic review was performed and described the evolution of tFUS research in humans along the years. No study in humans to treat depression was found. Neuronavigation was considered a major concern, especially for deep brain targets. Therefore, an opensource neuronavigation method was optimized to fit user-friendliness and to be adopted in a future clinical trial. To improve targeting procedures, a computational model of the International 10-20 System was studied to correctly place the transducer in real-time neuronavigation. Finally, a clinical trial protocol was established using the software and hardware available, leading to a pilot study with the first patient enrolled in the study.

Conclusions: The tFUS is a promising neuromodulation method. This thesis provides improvements in neuromodulation procedures and proposes a clinical trial to assess the safety and efficacy of repeated sessions for TRD. However, there is still a need of replication of these methods by different centers, as well as the assessment of safety and efficacy.

Keywords: transcranial focused ultrasound; treatment-resistant depression; neuromodulation; transcranial magnetic stimulation; neuronavigation.

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1. INTRODUCTION

1.1. Low-intensity transcranial focused ultrasound neuromodulation

Ultrasonic technology has been employed in clinical practice since 1947 when it was first used to produce images of living humans¹. Since then, these devices have found extensive applications, particularly in the field of diagnostic medicine. Over the past decade, there has been significant research into therapeutic ultrasound across various medical specialties, including physical therapy and oncology². These therapeutic interventions are often categorized as high-intensity (1,000 to 10,000 W/cm²), medium-intensity, or low-intensity (< 3 W/cm²), each having distinct mechanisms of action³. High-intensity ultrasound primarily relies on rising temperature for thermal ablation, whereas low-intensity ultrasound is believed to induce reversible cellular changes⁴.

Ultrasound, defined as sound waves with frequencies beyond the upper range of human hearing, is typically generated in clinical settings using continuous-wave or pulsed-wave modes, each with unique characteristics⁵. Continuous-wave ultrasound operates continuously, while pulsed-wave ultrasound is characterized by on-off cycles, with variations in on/off pulse ratios. Pulsed ultrasound is commonly used for non-ablative purposes to minimize thermal effects, which are proportionate to the on/off pulse ratio⁶.

In the context of transcranial focused ultrasound (tFUS) research, understanding how neuromodulation patterns relate to various stimulation parameters is a major focus. These parameters include acoustic frequency (Af), pulse period (PP), pulse repetition period (PRP), pulse repetition frequency (PRF), duty cycle (DC), and stimulus duration (SD). Additionally, when multiple sonications are performed, parameters like inter-stimulus interval (ISI) and total session time become relevant, though their nomenclature varies among studies. The ISI is usually measured in seconds and is considerably larger than the DC, mainly because of the safety profile of tFUS. Heat dissipation can happen via conduction, convection or other removal processes, and is expected to overcome theoretical heat production inside the brain. Therefore, the ISI provides time for heat dissipation after sonications, preventing undesired effects of thermal ablation in the brain⁵.

Safety concerns in low-intensity ultrasound interventions involve three crucial parameters: the mechanical index (MI), spatial-peak temporal average (I_{SPTA}), and spatial-peak pulse average (I_{SPPA}). Regulatory bodies like the FDA have established safety limits for these parameters in human neuromodulation studies, which are $I_{SPTA} \leq 720 \text{ mW/cm}^2$, $I_{SPPA} \leq 190 \text{ W/cm}^2$ and $MI \leq 1.9$ ⁷.

Regarding the specific effects of low-intensity focused ultrasound on neuromodulation, studies have shown its potential to modulate neuronal activity in both the brain and peripheral nerves^{8, 9}. While the exact mechanism remains uncertain, hypotheses include radiation force-induced deformations¹⁰ or cavitation effects on neuron cell membranes¹¹. Thermal effects are less pronounced in low-intensity ultrasound, but the attenuation of ultrasonic waves can lead to thermal energy deposition, potentially affecting neural activity^{12, 13}. Regardless of the mechanism of action of the modulatory effect of low-intensity focused ultrasound in the nervous system, it is considered to be transient and non-ablative¹⁴.

The unique acoustic properties of the brain, influenced by its water content, enable ultrasound waves to be focused across solid structures with minimal power loss^{5, 15}. This property makes ultrasound particularly attractive for neuromodulation, which aims to either enhance or inhibit neural activity in specific brain areas¹⁶. However, current clinical applications of neuromodulation are limited to invasive methods or cortical stimulation techniques¹⁷, restricting its use in treating neuropsychiatric disorders.

Transcranial focused ultrasound (tFUS) is an emerging technique designed to modulate neural signaling with high spatial resolution and deep brain stimulation capabilities. Unlike standard diagnostic ultrasound devices, tFUS devices have a concave shape that focuses acoustic energy. These devices may consist of a single element or multiple transducers that can be adjusted to control their distance from the target area.

Studies involving tFUS have demonstrated excitatory effects on various brain regions in animals¹⁸⁻²³, as well as inhibitory effects in others²⁴⁻²⁹. Achieving non-invasive neuromodulation in humans, especially in deep brain structures, poses challenges that require accurate neuronavigation techniques, as previously demonstrated in larger animals^{25, 30-33}. The debate over tFUS's bimodal effects on neuron activation centers on parameters like DC and SD, which appear to play a

significant role in determining whether tFUS facilitates or inhibits neuron firing³⁴. The involvement of T-type calcium channels in cell-type-selective neuromodulation is also a subject of interest, suggesting that different parameters may yield distinct neuromodulatory effects in different brain regions³⁵.

In some studies, tFUS has been combined with intravenous microbubble contrast agents, primarily aimed at opening the blood-brain barrier (BBB)^{36, 37}. This combination has the potential to enhance drug delivery to specific brain regions, including intranasal administration³⁸. The mechanism behind BBB opening during sonication with microbubble contrast agents is thought to involve microbubble oscillation and inertial collapse⁵, although the precise details remain unknown.

1.2. Comparison methods in tFUS studies

Low-intensity tFUS aims primarily at neuromodulation, which can be defined by either enhancing or inhibiting neuronal activity in specific brain regions¹⁶. Many methods were used in the past to evaluate the neuromodulatory effect of low-intensity tFUS. The main examples are: (1) behavioral, electroencephalogram (EEG), or functional change after baseline, using the patients as their own control group³⁹⁻⁴¹; (2) sham-control⁴²; (3) neuroimaging changes^{43, 44}; and (4) differentiation of outcomes using different tFUS parameters⁴⁵. Although these strategies are interesting for single sonications, their translation into clinical practice is limited due to the lack of comparators already approved for the treatment of mental disorders.

Among the other non-invasive neuromodulation techniques that could be used for comparison versus tFUS, the transcranial magnetic stimulation (TMS) must be highlighted. It has been extensively studied for neuromodulation^{46, 47}, with focus on major depressive disorder (MDD)⁴⁸⁻⁵⁰, and treatment-refractory depression (TRD)⁵¹⁻⁵³. TMS offers the advantage of being feasible in an outpatient setting without the use of anesthetics, in contrast with other neuromodulatory techniques such as electroconvulsive therapy (ECT)⁵⁴. However, its applicability is limited to cortical areas and cannot reach deep brain structures⁴⁷. Although one study has used both tFUS and TMS⁴², it has not compared both techniques, but joined them to evaluate possible attenuation of motor-evoked potential (MEP) by tFUS.

1.3. Potential of TMS as a comparator for tFUS studies

TMS offers additional advantages that highlight it as a potential comparator for tFUS. Typically, it is delivered in a repetitive setting of 10 Hz stimulations in once-daily sessions for 4 to 6 weeks⁵⁵. Although considered effective, this setting is considered time-consuming and new protocols have been studied in comparison with the classic TMS schedule^{47, 49, 50, 53, 55, 56}. One of these new approaches is the intermittent theta-burst stimulation (iTBS), which is classically composed of 3-pulse (50 Hz) bursts, delivered at 5 Hz in an intensity of 80% of the motor threshold⁵⁷. The benefits of iTBS include shorter sessions' length and a similar efficacy when compared with classic TMS protocols⁵².

Although these protocols differ in the treatment schedule and stimulation intensity, there is a relative consensus about the area to be targeted to maximize TMS's results⁵⁸: the left dorsolateral prefrontal cortex (L-DLPFC). It is a functional area placed in the middle and superior frontal gyri of the frontal left lobes. It has primarily been associated with "cognitive" and "executive" functions⁵⁹, which are usually impaired in MDD and TRD. Interestingly, recovery from depressive symptoms (especially the ones associated with cognitive disfunction) was previously associated with an increase in the L-DLPFC metabolic uptake of ¹⁸F-fluorodeoxyglucose using the positron emission tomography (PET) scan⁶⁰. These results raise the hypothesis that stimulating the L-DLPFC may be beneficial in the treatment of MDD and TRD.

The positioning of the L-DLPFC according to the skull area, however, reasoned an extensive debate in the scientific scenario, and currently a method called Beam F3 is the most widely used in clinical practice⁶¹. Although it is considered to be reliable for targeting the L-DLPFC, it is largely dependent on manual measurements and interpersonal differences. This has pressed research to carefully investigate the efficacy of the Beam F3 method^{62, 63}, and even to contest its previously established accuracy⁶⁴.

Currently, TMS has the closest clinical profile to tFUS, due to its low invasiveness, low risks of severe adverse effects and local neuromodulatory potential. Besides, it already carries within a brain volume target and a specific mental condition, which could possibly guide the targeting and protocol of tFUS.

1.4. MDD as a clinical condition for tFUS studies

MDD is considered a global health problem, affecting approximately 10% to 15% of the population annually⁶⁵. By definition, MDD is characterized by the persistence of 5 or more symptoms that cause functional impairment to the patient, highlighting mood depression and anhedonia⁶⁶. In previous studies, MDD's dysfunctional impact has already been compared to the ones caused by other non-psychiatric chronic diseases, such as diabetes mellitus and heart failure⁶⁷.

Pharmacological treatment is widely considered as an effective choice for the initial treatment of MDD^{68, 69}. However, about 30% of patients fail to achieve full symptomatic recovery even with multiple pharmacological attempts^{70, 71}, and are usually described as having TRD or another classification, called partially-responsive depression (PRD). These nomenclature definitions are critical for research guidance in the field, given the scarcity of standardization about the management of TRD and PRD. A recent consensus defined treatment response in MDD by a reduction of at least 50% in symptom severity, preferentially documented by psychometric assessments⁷². Although patients who fail to achieve such response are considered to be part of a pathological continuum, the definition of PRD and TRD is important to guide and standardize clinical trials and future research. Therefore, the same consensus defines PRD by a partial response (25% to 50%) to at least 1 antidepressant, while TRD is defined by a considerable lack of response (less than 25%) to at least 2 antidepressants. It is noteworthy that, for definition purposes, the antidepressant trial must be adequate both in duration and dosage.

1.5. Previous challenges of tFUS studies

There are three main differences between tFUS and TMS, which illustrate the challenging scenario of tFUS research. They are: (1) the mechanism of action; (2) the considerably different volume of stimulation, which is considered drastically smaller in tFUS; and tFUS's capacity of stimulating deep-brain regions. These characteristics give tFUS the status of a non-invasive, highly spatially specific, and deep brain reaching neuromodulation technique. Besides, skull attenuation of ultrasonic energy must not be neglected, since most authors consider the FDA limits at the site of stimulation rather than on the surface of the transducer.

Extensive research about such attenuation was conducted in the past, considering that a pressure map calculated on free water does not match the real

acoustic power deposited in the brain. In 2014, Legon et. al. recorded acoustic pressure in free water and through hydrated fragments of human cranium, concluding that I_{SPPA} drops about 75% after attenuation⁷³. Lee et. al. found similar values of 76% and 82% in different studies^{41, 74}. In a posterior study (2018), Legon et. al. conducted a computational simulation using CT models and found an attenuation of six- to seven-fold in intensity attenuation⁴⁰. These previous data allow researchers to increase acoustic power in the transducer and to assume at least 50% of skull attenuation⁷⁵. However, a recent study calculated different attenuations and found an attenuation of only 18.2% by the skull overlaying the L-DLPFC⁷⁶.

The deep-brain reaching and high spatial specificity of tFUS makes its placement way more challenging than TMS's. As both techniques are restricted to a specific brain volume, a procedure called neuronavigation is usually necessary to guide the stimulation towards a pre-specified target. However, unlike TMS's, tFUS's neuronavigation is less studied and much more relevant, due to its higher spatial specificity, deep brain reaching and narrower spatial focus of stimulation. As shown in previous studies, the area of peak tFUS stimulation is cigar-shaped⁴¹, with a diameter of 0.5 to 1 cm.

In practice, it means that minimal position change or tilt of the tFUS transducer relatively to the patient's head may result in drastic changes of the stimulated area underneath in the brain. For this reason, all low intensity tFUS studies that targeted deep brain regions in humans were either MRI-guided^{39, 77} or used a stereotactic neuronavigation software based on fiducial markers, attached to the patient's head and to the tFUS transducer^{40, 42, 44, 78-80}. Some studies have not used a neuronavigation software, but they were either limited to cortical stimulation or to a single-burst stimulation that lasted no more than a few minutes^{73, 81, 82}. For this reason, repetitive tFUS stimulation requires a more robust and detailed method of neuronavigation, to ensure that the same region will be sonicated throughout the treatment days.

Some studies have not used a neuronavigation software, but they were either limited to cortical stimulation or to a single-burst stimulation that lasted no more than a few minutes. For this reason, repetitive tFUS stimulation requires a more robust and detailed method of neuronavigation, to ensure that the same region will be sonicated throughout the treatment days. To the best of my knowledge, no such method has been described for open-source neuronavigation.

2. RATIONALE

The research momentum of low-intensity tFUS stimulation in humans justifies the studies detailed in the present thesis. The abovementioned stimulation studies started in 2014⁷³, exactly one decade ago, and so far have shown a promising profile of non-invasive deep brain stimulation with high spatial specificity. However, the literature still fails to provide a reproducible, feasible, and standardized method for translating such a promising theoretical technique into clinical practice. Moreover, most patients enrolled in tFUS stimulation studies so far were healthy, and the sonication only happened once. This raises concerns about the safety of repetitive stimulation protocols, neuropsychiatric conditions that may benefit from the technology, and what are the best practices for in-field practical stimulations.

Stimulation design protocols generally consider previous protocols⁸³, translational protocols tested in animals⁷³, and protocols whose test was previously described in humans⁸¹. Repetitive protocols with other neuromodulation techniques have been proven safe in humans with depression⁸⁴. On the other hand, the literature lacks a systematic review of all studies published so far that used the tFUS in low intensities to achieve neuromodulation in humans. Besides, although cortical low-intensity stimulation seems safe after a single sonication⁷⁷, no study has shown functional and morphological safety after repetitive sessions. Apparently, the point of maximum acoustic energy deposition suffers little thermal rise after a single sonication^{41, 85}, but it cannot be inferred for repetitive sessions. Therefore, the safety of repetitive stimulations must be addressed in a proposed systematic review of the theme. Such a study may help design safer and more reproductive protocols, considering data that have already been published. Therefore, a study review assessing all protocols in humans with tFUS to date is needed.

Achieving real-time neuronavigation is essential for tFUS stimulation. This is justified by the considerably larger volume of stimulation compared with animals and the relatively small, specific volume of stimulation achieved with the tFUS. So far, only one study describes an open-source mechanism for neuronavigation using the tFUS⁸⁶. However, the study did not undergo peer review, and its results might not fit the desired user-friendliness and practical applicability required by the clinical setting proposed in

this thesis. Therefore, a study to analyze and, if necessary, optimize the methods previously described by Preiswerk et. al.⁸⁶ is needed. Moreover, such a proposed analysis may raise additional requirements that are currently unavailable and justify further studies by itself.

All the information gathered in sections 1.2, 1.3, and 1.4 of this thesis must be considered to propose a clinical trial. Stimulating a cortical area is easier than aiming at a deep brain region, and TMS seems to be a reasonable comparator for a large tFUS study that analyses the safety and efficacy of tFUS in humans. Moreover, the impact of TRD mentioned in section 1.4 justifies the study of tFUS in this population, who needs additional therapeutic options. This, by itself, justifies a whole non-inferiority study of tFUS against TMS, using a similar protocol to address both the safety and efficacy of tFUS in patients with TRD.

Any clinical trial must be submitted for institutional ethical approval, and its variables must be exploited before initiating. However, a protocol study that undergoes rigorous peer review may help guarantee that the clinical trial will follow a specific method that is accepted by the scientific community. Therefore, before initiating such a clinical trial, a protocol study is more than welcome, and the peer-review process (which is frequently accompanied by a reviewer's response) may elicit details that the main researchers overlook. This also helps to avoid changes during the clinical trial, which may be beneficial to avoid several types of biases, such as selection, observer, and undercoverage biases.

Last but not least, proposing a study that aims at converging a novel technology into a reliable and reproducible clinical trial would be incomplete without a pilot test. It would provide the research community with the information that the proposed protocol can be achieved in real practice and is possibly replicable in a whole clinical trial.

3. OBJECTIVES

Primary objective: to develop a reproducible, clinically reliable methodology for low-intensity tFUS stimulation over the L-DLPFC in patients with TRD. Secondary objectives: (1) to perform a systematic review of the literature to describe the best practices and the heterogeneities of studies using low-intensity tFUS stimulation in humans aiming at neuromodulation so far; (2) to optimize the open-source

neuronavigation methods available so far for low-intensity tFUS stimulation in humans; (3) to study computational models to standardize the placement of the targets of stimulation; (4) to develop a protocol for studying the safety and efficacy of tFUS against TMS in the treatment of patients with TRD; and (5) to test the method in a pilot patient and describe preliminary data both about safety and efficacy of the stimulation and about practical challenges during stimulation sessions.

4. MATERIALS AND METHODS

Several methodological steps were needed to develop a reliable protocol to assess the safety and efficacy of low-intensity tFUS stimulation in patients with TRD. Since this thesis focuses on (but is not limited to) a protocol proposal, the methods needed to be rearranged after each step so that the results of the previous step helped to shape the structure of the subsequent methods. Therefore, unlike most research papers, this section will provide little explanation about the results of each step to set the reader on the timestamp of different methods adequately. Since the materials remained the same during the development of the protocol, they will be described in a separate subsection.

4.1. Materials

The available hardware was composed of (1) a Dell 8GB-RAM computer using Windows 10 version; (2) a MacBook M2, 8GB-RAM computer using MacOS Sonoma 14.4 version (3) an optical infrared-based stereotaxic camera NDI Polaris Vicra® used for real-time neuronavigation; (4) a Brainbox NeuroFUS PRO® V2.0 S/N 021 with CTX-500MR Compatible Transducer, calibrated in April 22nd 2022; (5) 3D-printed fiducial markers coupled to infrared-reflective spheres (as illustrated in section 5.2); and (6) continuously produced degassed water, whose degassing process was previously described⁸⁷.

The software used in this thesis were: (1) XQuartz version 2.8.5 (2) 3D Slicer version 5.2.2, with the extensions FiducialsToModelDistance, GeodesicSlicer, SlicerFreeSurfer, SlicerIGT and SlicerOpenIGTLink; (3) FreeSurfer version 7.3.1; (4) NDI 6D Architect Version 3; and (5) Plus Server Launcher Version 2.6.0.

Imaging tests available were (1) morphological MRI and (2) PET/CT scans, which were both used in this protocol. Supplementary File 1 provides additional detail about distinctive chairs, helmets, headbands to fix fiducial markers to the patients' heads, and office material to produce a stable environment for sonication.

4.2. Methods

This thesis' first step was understanding the current stages of research using tFUS in humans and listing the challenges and literature gaps so far, especially for depression. A systematic review was designed and performed to address this issue. The methods for this systematic review are thoroughly described in section 5.1. This review presented important data concerning neuromodulation protocols in humans, targeting strategies, and different efficacy and safety assessments. An essential concern collected in the systematic review was finding the exact point of maximum acoustic energy deposition during stimulation procedures. This raised the need for a neuronavigation method, which has become increasingly necessary.

While the hardware for neuronavigation was already available at the Centro de Tecnologia em Medicina Molecular (CTMM-UFMG), the use of neuronavigation software for tFUS was not available. Therefore, the second step was to test and improve an open-source neuronavigation method. So far, few methods were explicitly available, although some of them were not peer-reviewed⁸⁶. This method requires primary material available for the UFMG neuromodulation team and was chosen as the primary method for sonication for this reason. Some of this material is described in the "Materials" subsection. Although the method proved reproducible, its results did not reach the user-friendliness needed for a neuromodulation team with a high turnover rate. Therefore, an optimization was proposed and described in section 5.2., in which the specific methods are thoroughly described. This was considered the second step of this method.

Once the neuronavigation method was chosen and improved, there was a need to enhance the automatization of targeting. Although neuronavigation provided real-time visualization of a point inside of the brain (commonly named "focus"), a two-point method would maximize reproducibility across sections, as described in section 5.4. This need for a superficial target led to using the International 10-20 setting, the base of one of the most commonly used methods to guide TMS⁶¹. Therefore, this thesis's

third step is proposing an open-source computational model to increase the automatization and reproducibility of the superficial target setting. The specific methods are detailed in section 5.3.

Once multiple applicators mastered the neuronavigation method and gathered information concerning the current state of the art of tFUS research in humans, the know-how was considered enough to propose a clinical trial. This proposal was considered the fourth step of this thesis and is resumed and illustrated in section 5.4, which contains a paper that has already been reviewed by peers and published (DOI: <https://doi.org/10.1016/j.ultsonch.2021.105890>).

Once the protocol was defined, the pilot study could start. Since UFMG's outpatient clinic complex did not have a specific Depression Outpatient Clinic, it was necessary to design one. A specific preceptor was designated for the clinic, and the rotation in it was added to the Psychiatry Residency Program (Categorical) at UFMG's University Hospital during post-graduate year 3 (PGY-3). PGY-3 residents were responsible for assessing the patients referred to the hospital and were essential to the protocol, as specified in "blinding", a subsection of section 5.4. Since the Brazilian public healthcare system does not cover TMS and ECT was hard to achieve, the possibility of nonpharmacologic methods for treating refractory cases was the main attractive characteristic of the outpatient clinic. Therefore, it was named Refractory Depression Outpatient Clinic, and the possibility of referral was discussed with preceptors of different outpatient clinics across the UFMG's complex.

Since practical reliability and reproducibility are at the core of this thesis, a pilot study was conducted with the first patient sonicated using the protocol proposed in section 5.4. This was considered the fifth and final step of this thesis and provided feedback from both the patient and multiple applicators about refining the method. These details are provided in section 5.5 and are considered vital since practical experience provides insights that would not be available by theory alone.

In alignment with this narrative methodology, the results are presented as papers written either as to be published in the future or as already submitted. Between each paper, the advances and discoveries that led to the next will be discussed, and practical concerns will be detailed to align the presented papers with the practical workforce needed to optimize the theoretical principles described in the papers and applied in the clinical context. Since several tables and figures were required for each

study, they will not be presented in different files except when extraordinarily necessary. Supplemental material will be reserved for highly technical material and information that concerns the practical optimization of the method discussed in this study but does not belong to the body of the text. The references in all papers presented in this thesis will follow the order mentioned in the body of the text, and, therefore, the “References” section of each study will be removed. All references were standardized and are listed in section 8.

Lab notes were also discussed as a descriptive method of this thesis. They consisted, for example, of the most adequate positioning for the patient and the applicator, the practical challenges of neuronavigation, and the number of researchers needed for a single sonication. The objective is to transcribe mere observation (which is, as mentioned at first in section 1, frequently neglected in published research) into succinct, directed, and feasible information for future research. All efforts were made to produce, optimize, and describe a reproducible method that can be used for a proposed clinical trial and, if needed, translated into other research.

5. RESULTS

5.1. Systematic review

The first step in providing a reproducible method was to produce a systematic review (PROSPERO CRD42022378854) to provide state-of-the-art information about research on low-intensity tFUS stimulation in humans. It also helped to elucidate the practical aspects used so far, their possible limitations, and how sonication parameters were used in the past. The tFUS device available at UFMG allows the researchers to change specific parameters, illustrated in Figure 1 of Supplementary File 1. Therefore, any protocol proposed in this thesis would necessarily need to take into consideration data already published on how those parameters were set and what they represent. This study was submitted to Ultrasonography (eISSN 2288-5943, ID USG-24-074) and is under review to this date.

USE OF LOW-INTENSITY TRANSCRANIAL FOCUSED ULTRASOUND FOR NEUROMODULATION IN HUMANS: A SYSTEMATIC REVIEW

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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ABSTRACT

Purpose: to systematically review data about efficacy, safety, means of use, and outcome measures of neuromodulation using low-intensity transcranial focused ultrasound (tFUS) in humans. Methods: interventional original studies were included following Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The quality of the studies was assessed by the Risk Of Bias In Non-randomized Studies (ROBINS-I). Results: tFUS was used for neuromodulation in more than 200 individuals so far, with outcomes that include neuronal activity facilitation, inhibition, and mixed results. These neuromodulatory outcomes are related to different sonication protocols. A low risk of bias was considered in most of the studies. Conclusion: More studies concerning adverse events in long-term use and clinical applications are still necessary.

Keywords

Transcranial focused ultrasound; humans; neuromodulation; neuronavigation.

Keypoints

- Transcranial focused ultrasound was firstly tested in 2014 and is a promising method for neuromodulation in humans. Its profile has been considered safe so far in many studies.
- Most previous studies were conducted using a single sonication, which raises the need of further studies to understand the safety and efficacy profile of multiple sonications.
- The minimum setting for the focused ultrasound stimulation is the a transducer, a magnetic resonance image and a neuronavigation method, which has been improved over the years.

INTRODUCTION

Ultrasonic devices have been used in clinical practice since 1947, when the first ultrasonic image of a living human was recorded¹. Since then, the use of these devices has grown considerably, especially in the field of diagnostic medicine. In the last decade, therapeutic ultrasound has been investigated in many medical specialties, including oncology and physical therapy². These interventions are generally described as of “high-intensity” (1,000 to 10,000 W/cm²), “medium-intensity” or “low-intensity” (< 3 W/cm²), each with different known mechanisms of action³. While high-intensity acoustic waves are thought to predominantly act via temperature rise leading to thermal ablation, low-intensity ones probably exert their effects due to reversible cellular changes⁴.

Ultrasound is defined as a sound wave (which describes periodic pressure disturbances in a medium propagating through space and time) that oscillates at frequencies higher than the human audible range. In most clinical applications, the generation of ultrasound is typically divided into continuous-wave and pulsed-wave modes, each with its own characteristics⁸⁸. The main difference is that the ultrasound generation is always on in the former, and the waves are uninterruptedly delivered. In contrast, in the latter, the generation is carried out in “on” and “off” cycles. Although the time periods dedicated to “on” and “off” periods may vary, by definition, the cycles repeat them through a pre-determined duration. Therefore, pulsed ultrasonic waves can be conventionally described by their on/off pulse ratio and the number of cycles (or, interchangeably, the total duration of cycles). When therapeutic ultrasound is used for non-ablative purposes, it is usually delivered in a pulsed wave setting to reduce undesired thermal effects, which is considered to be approximately proportional to the on/off pulse ratio⁶.

One of the major interests in tFUS research is to understand how the neuromodulation patterns are related to different stimulation parameters, which are directly associated with the physical properties of the ultrasound beam. The nomenclature for such parameters still finds discrepancies within the literature. Nevertheless, they may be standardized as follows: the acoustic frequency (or fundamental frequency, commonly mentioned as A_f) corresponds to the delivered wave's frequency, expressed in Hz. The pulse period (PP), also named as “pulse duration” can be defined as the temporal width of an ultrasonic pulse, corresponding

to the smallest amount of time that a single ultrasound pulse remains on. The pulse repetition period (PRP) is defined as an elapsed time between the start of sequential pulses, corresponding to a whole on-off cycle. The pulse repetition frequency (PRF) is defined as the number of pulses that occur within one second, measured in Hz. PRP and PRF are indirectly proportional, and they can be also calculated through the formula $PRP = 1/PRF$. The Duty Cycle (DC) is the ratio between the time the signal stays on and the time it takes to complete an on-off cycle. Therefore, it is a measure of the fraction of time that the ultrasound pulse remains on though the cycle, being presented either as a percentage or a whole number (e.g., 0.5). It can be calculated as $DC = PP/PRP$. Finally, the stimulus duration (SD) corresponds to the whole time of sonication, and can be calculated as the number of cycles times PRP.

The parameters defined above can successfully describe a single sonication. However, additional measurements apply for repeated sonication and must also be standardized. If more than one stimulation is done, than one can also define an inter-stimulus interval (ISI), measured in seconds, which is usually considerably longer than the SD. One of the reasons for this practice is based on physical safety: a sufficiently long interval between stimuli prevents that heat production overcomes heat dissipation by conduction, convection or other removal processes, leading to harmful thermal rise of the brain tissue⁵. Finally, one can also mention the total session time, which corresponds to the period between the beginning of the sonication and its end, when the transducer is distanced from the patient's head. It is noteworthy that these names vary considerable among studies, due to lack of standardization parameters. The nomenclature standardization of the parameters in the analysis of tFUS stimulation in this systematic review is illustrated in Figure 1, as well as the main components involved in tFUS sonication.

Three other parameters are also fundamental for preparing and reporting low-intensity ultrasound interventions due to safety concerns. They are the mechanical index (MI), spatial-peak temporal average (I_{SPTA}), and spatial-peak pulse average (I_{SPPA}). The MI is defined as the derated peak rarefactional pressure (measured in MPa) divided by the square root of the center acoustic frequency⁵. I_{SPTA} is calculated by the pulse intensity times the PRF, and I_{SPPA} is calculated by the pulse intensity divided by the PP⁸⁹. The United States Food and Drug Administration (FDA) has set

limits for $I_{SPTA} \leq 720 \text{ mW/cm}^2$, $I_{SPPA} \leq 190 \text{ W/cm}^2$ and $MI \leq 1.9$ ⁹⁰, which are generally respected in neuromodulation studies with humans.

Regarding the specific neuromodulatory effects of tFUS, ex-vivo studies have shown that ultrasonic waves can be used to modulate neuronal activity in the brain and in peripheral nerves^{8, 9}. Although the exact mechanism is still unknown, it has been hypothesized that it is related to deformations caused by radiation force¹⁰, or cavitation between the layers of the neuron cell membrane¹¹. The effects of thermal rise in neuromodulation are still unknown, since low-intensity ultrasound is thought to elicit little temperature variation, in contrast with high-intensity applications. However, the attenuation of the ultrasonic wave invariably leads to thermal energy deposition¹², which might not be neglected, since even slow thermal variations may cause modulation of neural activity¹³. Regardless of the mechanism of action of the modulatory effect of low-intensity focused ultrasound in the nervous system, it is considered to be transient and non-ablative¹⁴.

The acoustic properties of the brain are strongly influenced by the composition of its biological tissue. Because of the distinctive amount of water in the brain, many of its physical properties are similar to that of water⁵ and, as in other soft tissues, ultrasound waves can be focused across solid structures with minimal power loss¹⁵. Furthermore, ultrasonic waves can be focused to small volumes (in the scale of millimeters) and concentrate acoustic energy in deep intracranial structures⁹¹. These characteristics are particularly interesting for neuromodulation, which is defined by either facilitation or inhibition of neural activity in a specific area¹⁶. Neuromodulation in clinical applications is usually restricted to methods that are either invasive or limited to the cortical surface¹⁷, limiting its current role in the treatment of neuropsychiatric disorders.

The transcranial focused ultrasound (tFUS) is an emerging technique that aims to either facilitate or inhibit neural signaling in a small area of the brain, with high spatial resolution and possibility of deep brain stimulation. It differs from usual diagnostic ultrasound devices due to its concave shape, designed to focus the acoustic energy instead of spreading it. A tFUS device may be composed of a single element or multiple ones, the last being able to combine different transducers to control their distance from the sonicated area (illustrated in Figure 1).

Sonication with tFUS has shown excitatory effects when applied to the motor cortex¹⁸, somatosensory cortex¹⁹⁻²¹, midbrain²², and cerebellum²³ of mice. Inhibitory effects were shown in the sonication of the thalamus and visual cortex in rats^{24, 92} and of the thalamus of swines²⁵. It has also shown inhibitory effects in animal models of seizures and epileptic bursts in electroencephalogram (EEG) in mice, rats and macaques²⁶⁻²⁹.

Non-invasive neuromodulation, especially targeting deep brain structures in humans, might be challenging. Neuronavigation is the process by which one can accurately target an area in the brain, in real-time, for non-invasive neurostimulation. In studies with tFUS in small animals, the targeting is based on prior knowledge about the brain architecture and its surrounding superficial structures. However, in larger animals, the spatial specificity of tFUS makes neuronavigation increasingly more complex, which has been shown in previous studies with animals^{25, 30-33}. It is deductible, therefore, that human stimulation with tFUS might need robust methods for neuronavigation, which has been developing rapidly in the past years.

The bimodal effects of tFUS in neuron activation has been subject of debate among studies. A study conducted by Yoon et. al. suggests that sonication parameters (especially DC and SD) play a major role in determining whether the tFUS inhibits or facilitates neuron firing³⁴. It has also been theorized that T-type calcium channels are particularly important for cell-type-selective neuromodulation³⁵, which could suggest that a sonicated area may theoretically contain both inhibited and stimulated neurons. Although this subject still lacks more robust evidence, this theory also suggests that the sonication parameters may result in different neuromodulatory effects according to the sonicated area.

Sonication with tFUS was also evaluated with intravenous microbubble contrast, whose main medical utility is to increase the echogenicity of blood⁸⁸. However, in contrast with neuromodulation, most previous studies that combined microbubble contrast with tFUS sonication did so aiming blood-brain barrier (BBB) opening^{36, 37}. It has also been studied that this combination may help drug delivery in specific regions of the brain, including those applied via intranasal route⁹³. Although the exact mechanism by which microbubble contrast leads to BBB opening when subjected to sonication is unknown, it is usually explained by microbubble oscillation due to mechanical energy. With sufficient mechanical pressure, this oscillation may cause the

bubble to collapse, a phenomenon called “inertial collapse”⁸⁸, opening the tight-junctions of the BBB.

Although this pivotal field is growing and more sophisticated devices are being tested, the methods for neuromodulation with tFUS in humans still lack standardization and guidelines focused on best practices. Therefore, a standardized and systematic analysis of the theme is needed to best guide future research, although non-systematic reviews have partially covered the theme in previous studies and deserve mention^{14, 17, 38}.

This systematic review aims to investigate the role of tFUS for neuromodulation in humans.

MATERIALS AND METHODS

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist⁹⁴. The protocol was developed in December 2022 and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) in December 2022 (CRD42022378854).

Search strategies and study design

The search was performed through the Medline (Medical Literature Analysis and Retrieval System Online/PubMed), Scopus and Lilacs databases. Studies published up to December 2022 were included. The key terms used were “transcranial focused ultrasound” and “human”. Studies in Portuguese, Spanish and English were selected. The manual search of texts was performed by two independent reviewers (G.D.S.A.M. and G.C.G.), who analyzed the titles and abstracts; afterward, texts that fulfilled the requirements were included for further analysis. Any discrepancies between the reviewers were resolved by decision of a third reviewer (B.M.V.).

Any review that was excluded in the primary search was included in a parallel analysis that used the “snowball method” to reach texts that were possibly missed. This method consisted in pooling all the references cited in the reviews and analyzing the titles and/or abstracts (when available) of all non-duplicate references. The studies that fulfilled the eligibility criteria were included in the final analysis, which was also

performed by two independent reviewers. It consisted of the full reading of the texts, exclusion of the ones that did not met the eligibility criteria and final data extraction.

Eligibility criteria

The inclusion criteria were: (1) original interventional studies that used low-intensity tFUS for neuromodulation; (2) case series, case reports or letters to the editor that mentioned the use of tFUS; and (3) studies performed in humans. The exclusion criteria were (1) reviews or meta-analyses; (2) studies that had not described any sonication in humans; (3) studies that used non-transcranial or non-focused ultrasounds; (4) studies that did not clarify if the device used was transcranial or focused; (5) studies that were not performed in humans; (6) retrospective studies; and (7) studies that used tFUS for other purposes, such as surgical ablation or BBB opening with microbubble contrast. Studies that used intravenous microbubble contrast were excluded to reduce the confusion bias of this systematic review, which is aimed at analyzing the direct effect of tFUS sonication in neuronal activity. As tFUS is a novel technology and this review aims to gather information about the maximum number of patients that underwent sonification via tFUS, the absence of control groups was not an exclusion criterion.

Data extraction and quality assessment

All extracted data included information about publication (including author name and year of publication), sample characteristics (previous illnesses, male/female ratio, handedness, age, number of participants), intrinsic study information (including outcomes and measurements used), neuronavigation protocol, tFUS settings, summary of results and safety information.

The data extraction followed a Cochrane model⁹⁵ in which one of the reviewers (G.D.S.A.M.) extracted data and another (G.C.G.) verified them. If any disagreement was found, a third reviewer (B.M.V.) also verified the extracted data.

Quality assessment was performed by two independent reviewers (G.D.S.A.M and R.O.D.P.Q.F.) using the Risk Of Bias In Non-randomized Studies (ROBINS-I) tool⁹⁶. This tool was purposefully chosen considering that studies with tFUS in humans would probably not be randomized, due to the relative novelty of the technology. If any disagreement was found between the two reviewers, a third one (B.M.V.) also verified

the extracted data. The bias was analyzed in the 7 domains of the tool (due to confounding; in selection of participants into the study; in classification of interventions; due to deviations from intended interventions; due to missing data; in measurement of outcomes; and in selection of the reported result). The results were divided in low, moderate, serious, or critical risk of bias.

RESULTS

The initial search of databases revealed a total number of 149 non-duplicate studies. Nineteen original articles were included for full text assessment and 22 reviews were enrolled in the snowball method, which found 1,212 non-duplicate records. After application of eligibility criteria, 32 texts were included via the snowball method, totaling 51 full articles assessed for eligibility. Of them, 18 were included in data extraction^{39-45, 73-75, 77-82, 85, 97}. The PRISMA flow diagram is summarized in Figure 2.

The first transcranial sonication in humans with tFUS was made in 2014⁷³. Until December 2022, more than 200 individuals were submitted to tFUS neuromodulation, that aimed both cortical regions and deep brain structures. The brain regions studied so far included the somatosensory areas S1^{45, 79, 85} and S2⁸⁵, the motor area M1^{42, 43}, the visual area V1⁷⁴, the international 10-20 EEG region CP3^{73, 82}, the right inferior frontal gyrus⁸¹, the temporal lobe⁷⁷, the left hippocampus⁸⁰, the thalamus^{39, 40, 78} and the medial prefrontal cortex⁷⁵. Figure 3 illustrates the brain areas targeted for sonification in studies with humans so far. Most research was focused in demonstrating the potentials of tFUS for neuromodulation in healthy subjects aged from 18 to 65. Neurophysiological parameters such as EEG variations, MRI blood oxygenation level dependent (BOLD) signal changes, and clinical outcomes such as mood changes and safety were evaluated. The data extraction is detailed in Table 1.

Neuronavigation

Neuronavigation was a major concern in the studies included in this systematic review, reasonably due to the high spatial specificity of tFUS and its deep reach within the brain. Most studies that used neuronavigation opted for the MRI as the primary source of image acquisition, mainly because its widely recognized gold-standard status in displaying soft tissues. Although some studies used a dedicated software for tFUS

guidance (including imaging methods), others have observed the neuromodulation effects even without such robust neuronavigational specificity, placing the transducer over EEG sites^{73, 81, 82}. MRI-guidance was used for most studies, either coupled with a neuronavigation software or alone³⁹.

The neuronavigation software used in some of the included studies is described in Table 1. When MRI was used alone, without a software, the sonication was performed placing the transducer inside the MRI head coil, as described by Lee et. al. (2016)⁷⁴. Monti et. al. (2016)³⁹ also describes a “trial-and-error” positioning method of the tFUS transducer using the MRI, mentioning successful positioning after 3 trials. This same method was used by Stern et. al. (2021)⁷⁷, which mentions multiple repositioning of the transducer until the desired region was demonstrated by the MRI.

The use of the international 10-20 system for EEG electrode position was also used, especially in the earlier studies of tFUS in humans. However, all the studies that targeted deep brain structures were MRI-based, which limited EEG guidance to cortical studies only. The latest study included in this review that used EEG guidance mentioned it as a limitation, even though its target was cortical⁸¹.

Outcomes

The definition and measurement of neuromodulation efficacy was also challenging in the studies so far. The main outcome for each study is detailed in Table 1. The method most used for characterizing changes in neural firing under tFUS sonication was the EEG^{40, 41, 45, 73-75, 79, 82, 97}, followed by the functional MRI^{43, 44, 74, 81}. Multiple clinical and behavioral outcomes were used as a mean of neuromodulation definition, such as sensory detection without external stimulation^{41, 74, 85}, reduction of pain threshold⁷⁸ and even recovery from a comatose state³⁹. In studies that included behavioral or clinical outcomes, the validated scales used were the Coma Recovery Scale-Revised^{39, 98} and the Visual Analogue Mood Scale^{81, 99}.

Targets of sonication

Figure 3 illustrates the previously sonicated areas in studies included in this systematic review. The first area that was targeted was the primary sensory cortex in healthy patients. The studies of Legon et. al.⁷³ and Mueller et. al.⁸², both in 2014, targeted the left somatosensory cortex using the 10-20 system CP3 EEG electrode

position. They described EEG differences measured by nearby electrodes, and, therefore, demonstrated neuromodulatory activity. In chronological order, the study of Lee et. al.⁴¹ was the first one to mention a “image-guided tFUS navigation software that was developed in-house”, using MRI to guide a bilateral sonication that elicited sensory detection without external stimulation. It was also the first study to mention the use of fiducial markers to guide neuronavigation, whose relevance will be discussed further. The study conducted by Ai et. al. (2016)⁴³ focused on functional MRI changes, which are detailed in Table 1. In 2016, a study conducted by Lee et. al.⁸⁵ used MRI-guided system with fiducial markers to sonicate the primary and secondary sensory areas and found a high percentage of sensory detection without external sensory stimulation, after 20 sonication bursts. Julien et. al. (2020)⁹⁷ studied the EEG changes of different settings of tFUS sonication over the sensory cortex after median nerve stimulation. In 2021, a study conducted by Liu et. al. stimulated the sensory cortex using an optical-based neuronavigation software to understand the differences in the detection of two-point sensory stimulations by healthy subjects.

Following the chronological sequence, the second area to be sonicated was the motor cortex, by Ai et. al. (2016)⁴³, in the abovementioned study. The second study to stimulate the motor cortex was conducted by Legon et. al. (2018)⁴², coupling the tFUS transducer to a transcranial magnetic stimulation (TMS) coil to attenuate TMS motor evoked potentials. Further, Ai et. al. (2018)⁴⁴ used a stereotactic neuronavigation system to stimulate specifically the dominant thumb representation in the motor cortex, using a functional MRI to determine the spatial specificity of tFUS. In 2021, Yu et. al. used an optical-based neuronavigation system to stimulate the left primary motor cortical region representation of the leg and reported tFUS dose-dependent modulation of voluntary movement cortical activity using EEG. The third area studied was the visual cortex, by Lee et. al. (2016)⁷⁴. Using MRI guidance without a specific software, this study reported subjective perception of phosphene, EEG and functional MRI alterations after sonication.

Other areas were further on targeted. The thalamus was sonicated by Monti et. al. (2016)³⁹, which found a significant recovery from a comatose patient. Other studies to target the thalamus were the ones conducted by Legon et. al. (2018)⁴⁰ and Badran et. al. (2020)⁷⁸, both finding significant reduction in sensory threshold in sonicated patients compared to sham. The right inferior frontal gyrus was targeted by Sanguinetti

et. al. (2020), which found differences in affect and vigor 30 minutes after sonication. This study also shown different patterns of connectivity of the sonicated area with different regions of the brain, as detailed in Table 1. The left hippocampus, the anterior temporal lobe and the bilateral medial prefrontal cortex were also stimulated in recent studies^{75, 77, 80}.

Safety

A major concern of the studies included in this systematic review was to keep the intensity of the stimulation inside the safety parameters recommended by the FDA. This is specifically challenging in transcranial stimulation, due to the high attenuation that the skull bone may cause in ultrasound intensity. Therefore, most studies rely on empirical data that simulates this attenuation, computational simulations, or simply previously available data. Legon et. al. recorded acoustic pressure in free water and through hydrated fragments of human cranium, concluding that I_{SPPA} dropped about 75% after attenuation⁷³. Lee et. al. found similar values of 76% and 82% in different studies^{41, 74}. In a posterior study, Legon et. al. conducted a computational simulation using CT models and found an attenuation of six- to seven-fold in intensity attenuation⁴⁰.

While some studies measured the acoustic intensity parameters in free water, others placed a human skull between the hydrophone and the transducer to accurately calculate the intensity of tFUS stimulation with the skull's attenuation. Some studies considered previous data measurement to estimate a fixed attenuation in the target area. However, not all studies properly clarified these parameters, which are shown in Table 2. There were also significant differences in data reporting, most probably due to the expected attenuation of acoustic energy by the human skull.

Twelve studies mentioned safety as one of their outcomes, and one of them focused on safety as the primary one. Moderate and severe adverse events were not reported, but complaints about hearing from the tFUS transducer was observed⁸¹. One study sonicated patients with temporal lobe epilepsy with intensities up to 5,760 mW/cm² before undergoing an anterior temporal lobe resection. Afterwards, they evaluated the safety of tFUS by histopathological analysis of the resected tissue and found no evidence of apoptosis or tissue damage⁷⁷. Two studies estimated the thermal effects of tFUS sonication in the brain tissue, resulting in 0.007°C⁴¹ and 0.0092°C⁸⁵.

The thermal rise of the transducer surface was measured by another study using fiber optic temperature probes, resulting in a maximum rise of 1°C in a period of 20 minutes⁴³.

Sonication parameters

Most studies included in this systematic review analyzed the effects of tFUS on healthy patients. Both facilitation and inhibition of neural activity were found, but the sonication protocol varied significantly among studies. Two articles were specifically designed to use an “excitatory protocol” and a “suppressive protocol”, but none of them were conclusive whether these parameters differences resulted in the expected neuromodulatory effects. One of them was specifically designed to assess histological safety of tFUS⁷⁷ and the other one tested these protocols in the bilateral medial prefrontal cortex, which resulted in different patterns of EEG changes compared to each other and to SHAM stimulation⁷⁵. The sonication paradigm used in each study, as well as the explicit or implicit outcome, is described in Table 2 using the standardization defined by Figure 1.

Quality assessment

Quality assessment using the ROBINS-I Toolkit for Nonrandomized Studies is summarized in Figure 4. Most studies were considered as having a low risk of bias overall, but 8 studies were considered as having a moderate risk of bias. Only 1 study was considered to have a moderate risk of bias in “selection of participants into the study”, justifiably because only patients with severe temporal lobe epilepsy were included. The most common risk of bias was seen in the domain “due to missing data”. None of the studies analyzed by this systematic review was considered to have a serious risk of bias.

DISCUSSION

In this systematic review, the use of tFUS in humans was summarized and analyzed. To the best of our knowledge, this is the first review to systematically evaluate this specific type of stimulation, which needs standardization and guidance towards best practices for futures interventional studies in humans. Besides, the transducer used in tFUS is different from the one used in diagnostic medicine, which

justifies studies that analyze specifically this type of device. A recent systematic review was conducted to evaluate the use of unspecific ultrasound in neuromodulation¹⁰⁰, but the tFUS demands a specific approach due to its particularities.

As expected, most of the studies included in this systematic review were non-randomized, given the relative novelty of tFUS use for neuromodulation in humans. Most of the patients enrolled were healthy and most of the sonicated areas so far were cortical, although deep areas of the brain were already included in studies using tFUS in humans. The characterization of neuromodulation ranged from image studies, EEG and behavioral changes, and used both control groups and/or contralateral side comparison.

Interesting interventional studies were cut-off from this systematic review due to usage of unfocused ultrasonic devices. A remarkable example is the pivotal experiment conducted by Hameroff et. al., which concluded that brief sonification with a standard clinical ultrasound could lead to improvement in mood in humans¹⁰¹. Other studies did not make clear that they were using a focused device¹⁰²⁻¹⁰⁵, and, therefore, were excluded from this systematic review due to previously defined inclusion criteria. However, it is noteworthy that the results of these studies, despite being out of the scope of this review, reinforce the hypothesis that ultrasonic waves can modulate neuronal activity.

It is a relative consensus that neuronavigation plays a major role in tFUS stimulation in humans. It is especially necessary in the stimulation of deep brain areas, due to the spatial resolution of tFUS. Most studies analyzed in this review used MRI-guided optical-based neuronavigation, which is based on fiducial markers placed in both the patient and the transducer. Their spatial position is read by a camera and is computed by specific neuronavigation software, which informs in real time the position of the tFUS focus. For this process to work, neuroimage is fundamental and generally consists of brain MRI. However, some of the studies analyzed also used prior knowledge about surface references to guide the tFUS beam, mainly using EEG markings. This method, however, was restricted to studies that stimulated cortical regions, and have not been used by any deep-brain stimulation with tFUS.

The studies included in the review show a clear evolution of neuronavigation over the years: the earlier studies targeted only cortical regions and used the international 10-20 EEG system, which was gradually replaced by MRI-guidance.

Further on, a study that targeted a cortical region pointed the lack of MRI-guidance as a limitation⁸¹. This leads to the conclusion that MRI is almost mandatory for future tFUS research, which may be challenging due to unavailability or the high cost of MRI. Besides, neuronavigation software may also be costly, although one study mentioned the use of an open-source method of neuronavigation⁸⁰.

Since high-intensity ultrasound acts via thermal ablation and ultrasound is known to deposit thermal energy in soft tissues, safety concerns are a consensus among tFUS researchers. None of the studies included in this review reported serious or permanent adverse events, which is aligned with the hypothesis that low intensity tFUS is relatively safe for neuromodulation. A recent systematic review that aimed to evaluate safety of tFUS was aligned with this hypothesis, finding that most studies in animals and humans pointed to a favorable safety profile of tFUS⁷. Most of the studies in animals that resulted in adverse events (mainly microhemorrhages) were performed with ultrasonic intensities above the ones established by the FDA, suggesting that these guidelines are a reasonable starting point to establishing more robust evidence of safety for tFUS stimulation.

It is noteworthy that skull attenuation plays a significant role in determining the acoustic power used in tFUS application in humans. In a recent study, Brinker et. al. measured both acoustic power loss and focus distortion targeting multiple focuses after skull attenuation using direct hydrophone measurements and a real skull¹⁰⁶. It has shown a wide variety of spatial peak pressure reduction (from 18.2% to 69.6%), as well as in focus distortion, depending on the sonicated area. These variations were attributed mainly to skull bone thickness, skull curvature and porosity attenuation effects. This may explain, in parts, the variety of attenuations measured or calculated by the studies analyzed in this review.

Interestingly, the study of Stern et. al. reported a single case of acidophilic neurons and extravasation in the sonicated tissue⁷⁷. However, the authors also point out that these changes were present in the non-sonicated tissue and that the resection material was damaged during resection, making unclear if these findings are due to tFUS sonication or mechanical damage.

Whether the tFUS exerts its effects via inhibition or facilitation of neuron activity still needs extensive debate. However, most of the results in this review support the previous paradigm stated by Yoon et. al. that inhibition is achieved by low duty cycles

and longer sonication durations, in contrary of excitation³⁴. The two studies included in this review that performed this protocol in humans used the same paradigm, although none of them was conclusive about its reliability in the human brain^{75, 77}. However, studies that used either the facilitation or the inhibition protocol apparently present coherent results. The most remarkable exception was seen in the study of Liu et. al., which showed that the stimulation using a duty cycle of 6% during 5ms actually increased cortical excitability instead of inhibiting it⁷⁹, although this mechanism is up to debate.

As discussed in the methods session, this systematic review did not include studies that used intravenous microbubble contrast agents. However, two studies from Jeong et. al. are worth mentioning^{107, 108}. Both concluded that the ¹⁸F-fluoro-2-deoxyglucose positron emission tomography (PET) was successful in identifying sonicated areas via differences in the regional cerebral metabolic rate of glucose. This finding was statistically relevant even when though there was no evidence of BBB opening, which is noteworthy because none of the studies included in this systematic review used the PET scan for evaluating the efficacy of neuromodulation. One of the studies reported improvement in measures of memory, executive, and global cognitive function¹⁰⁷ and the other study correlated the changes in regional cerebral metabolic rate of glucose with clinical outcomes¹⁰⁸. This suggests that, when combining intravenous microbubble agents with tFUS, the PET scan may be useful in predicting response to treatment, although much more evidence on the field is necessary. In both studies, no adverse effects were mentioned.

Although the studies of Jeong et. al. did not show BBB opening with intravenous microbubble contrast, others did. Abrahao et. al. showed that four patients with amyotrophic lateral sclerosis presented an immediate increase in normalized gadolinium signal intensity ratio, which returned to baseline in the 24-hour follow-up¹⁰⁹. Rezai et. al. found similar results in the hippocampal sonication of six patients with Alzheimer's disease, with simultaneous microbubble injection¹¹⁰. These results indicate that, although tFUS may cause BBB opening, this effect is transient and probably limited by the first 24 hours post-sonication.

Another subject of interest in tFUS neuromodulation research is the feasibility and safety of multiple sonication sessions, which is generally required in other clinical neuromodulation techniques. In most studies included in this systematic review, either

data about this was unavailable or the methods consisted of single pulses of sonication. One study aimed the development of a protocol for device usage on human subjects, which was described as 140 seconds sessions delivered biweekly for three weeks, and reported no adverse effects⁸⁰. The maximum time of treatment duration was found in the study of Kim et. al., which sonicated patients for 20 minutes with a pulsed protocol⁷⁵. One excluded study used a transcranial ultrasonic device placed on the F8 EEG site for five days in depressed patients within a seven-day period, with no major adverse events described¹⁰².

Given the progress of low-intensity tFUS research for neuromodulation in humans, it is time to assess clinical effectiveness and safety in clinical populations with mental and neurological disorders. As most neuromodulation therapies are done using multiple sessions, studies that aim repetitive tFUS stimulation are necessary to establish both the safety of this novelty and its clinical reliability. Future research should also focus on patients with mental disorders, due to previous positive results in achieving remission or response with other neuromodulation techniques^{111, 112}. In this stage, it is also recommended that more robust and validated scales are used for measurement of clinical outcomes, since only two studies in this review used them.

Interestingly, tFUS was already used, at higher intensities, in the treatment of obsessive-compulsive disorder and major depressive disorder^{113, 114}. However, the mechanism of action of tFUS varies according to the intensity of application, and these studies aimed at irreversible thermal capsulotomy. Differently, the application of low-intensity tFUS for neuromodulation purposes (which is the focus of this systematic review) aims at reversible, non-thermal and non-ablative effects, which are core characteristics of clinically used neuromodulation techniques, such as the TMS⁴⁷. This puts the tFUS as a promising neuromodulatory technique for future psychiatric treatment, since it is non-invasive and has unique properties of deep-brain stimulation, high spatial resolution and seemingly favorable safety profile.

Some studies included in this review already suggest that low-intensity tFUS may be useful in mental disorders, such as the one conducted by Sanguinetti et. al. (2020)⁸¹. In his article, the primary outcome was enhancement of global affect and vigor using a standardized scale in healthy patients, suggesting that the neuromodulatory effects of tFUS may be useful in mood disorders. Other studies using non-focused ultrasound also reported mood improvement¹⁰¹ and symptom relief in

depressed patients¹⁰². In the case of future research aimed at clinical outcomes, however, it is fundamental to pre-establish the effect of tFUS in core areas of interest^{115, 116} and the protocol of stimulation, demanding more pre-clinical research and pilot studies.

Quality assessment was a major concern in this study, notably due to the need of standardization for further studies. Most cases of “moderate risk of bias” in the overall result judgement were due to missing data, which is considered critical for providing evidence-based background for new studies using low-intensity tFUS in humans. Although noticeable, this is considered justifiable due to the relative novelty of the technology and the lack of previous standardization about which parameters must be reported in such studies.

This systematic review summarized the main methods, outcomes and measurements made so far with tFUS neuromodulation studies to date. Initiatives are developing protocols for future research, such as the International Transcranial Ultrasonic Stimulation Safety and Standards (ITRUSST), which has, to date, short-term and medium-term goals to guide future research using tFUS in humans. It also points to the need to establish safe and standardized protocols for tFUS research in clinical settings, as well as to better understand the outcomes of tFUS neuromodulation, especially in those with mental disorders.

CONCLUSIONS

tFUS has been safely and effectively used as a non-invasive, deep-reaching neuromodulatory technique in humans. Although its neuromodulation capacity is well established, its underlying mechanism is still unclear and there is few evidence that supports the adequate protocol to achieve either excitation or inhibition with tFUS. Most studies using tFUS for neuromodulation were held within the intensity limits previously determined and showed no to little adverse events. The overall quality analysis of the studies using tFUS for neuromodulation in humans is considered good, although more data detail is necessary to decrease the risk of bias. Further studies, including randomized and sham-controlled settings, are advised to better understand the potentials of tFUS neuromodulation in humans.

FIGURE 1.

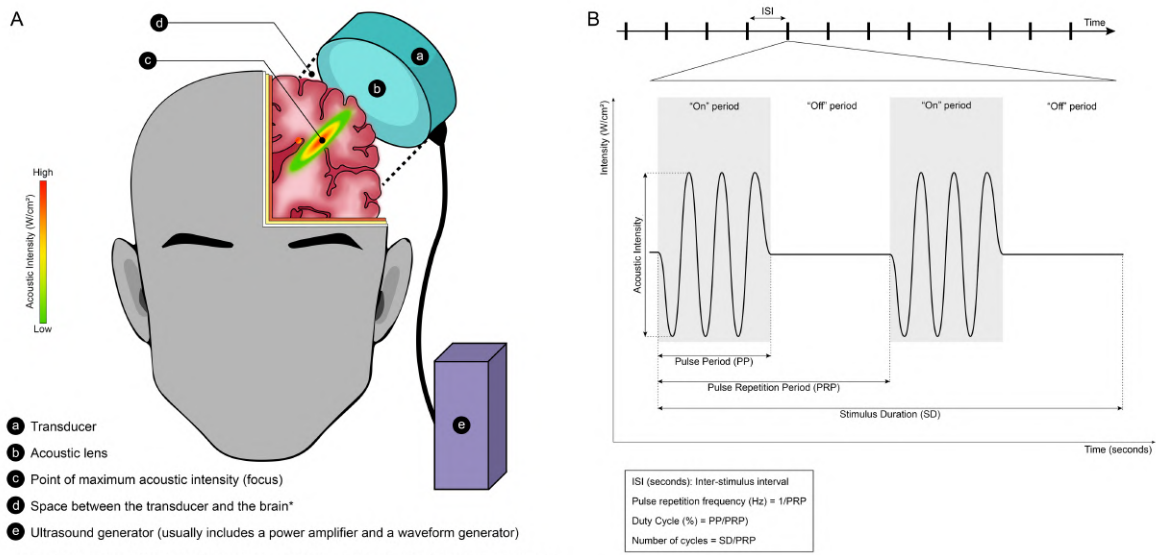


FIGURE 2.

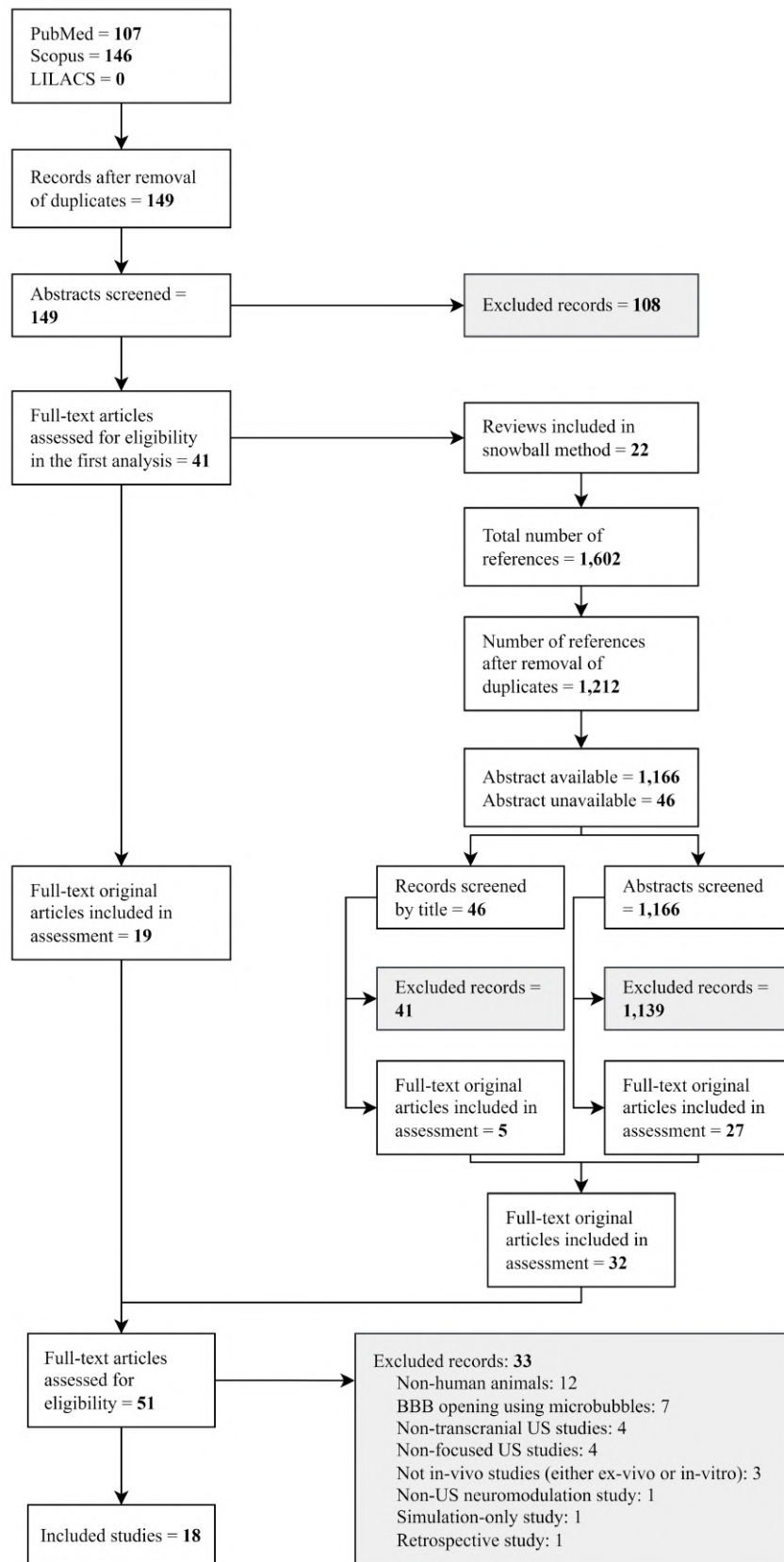


FIGURE 3.

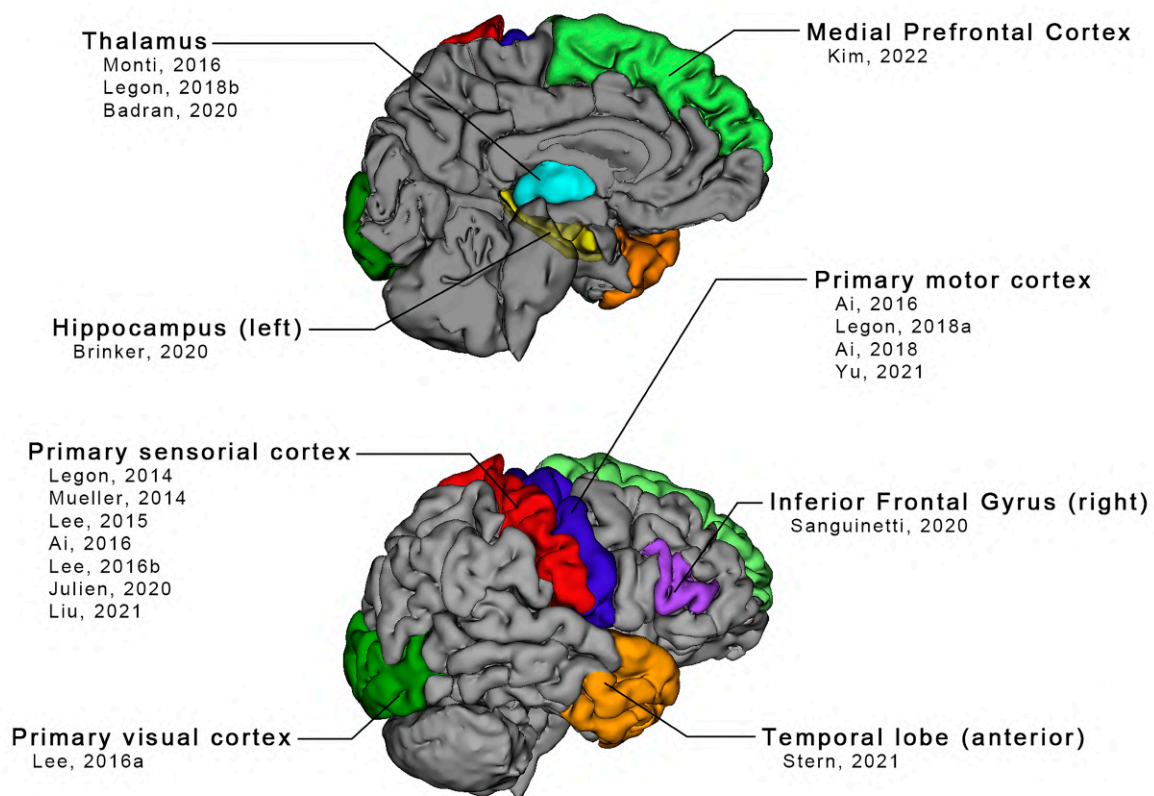


FIGURE 4.

	Risk of bias domains							Overall
	D1	D2	D3	D4	D5	D6	D7	
Mueller J et. al., 2014	+	+	+	+	-	+	+	-
Legon W et. al., 2014	+	+	+	+	-	+	+	-
Lee W et. al., 2015	+	+	+	+	+	+	+	+
Al L et. al., 2016a	+	+	+	+	-	+	+	-
Lee W et. al., 2016a	+	+	+	+	+	+	+	+
Lee W et. al., 2016b	+	+	+	+	+	+	+	+
Monti, 2016	+	+	+	+	-	+	+	-
Legon W et. al., 2018a	+	+	+	+	+	+	+	+
Legon W et. al., 2018b	+	+	+	+	-	+	+	-
Al L et. al., 2018	+	+	+	+	+	+	+	+
Sanguinetti JL et. al., 2020a	+	+	+	+	+	+	+	+
Sanguinetti JL et. al., 2020b	+	+	+	+	+	+	+	+
Badran BW et. al., 2020	+	+	+	+	-	+	+	-
Julien L et. al., 2020	+	+	+	+	+	+	+	+
Brinker ST et. al., 2020	+	+	+	+	-	+	+	-
Yu K et. al., 2021	+	+	+	+	+	+	+	+
Stern JM et. al., 2021	+	-	+	+	+	+	+	-
Liu C et. al., 2021	+	+	+	+	+	+	+	+
Kim YG et. al., 2022	+	+	+	+	+	+	+	+

Study

Domains:
D1: Bias due to confounding.
D2: Bias due to selection of participants.
D3: Bias in classification of interventions.
D4: Bias due to deviations from intended interventions.
D5: Bias due to missing data.
D6: Bias in measurement of outcomes.
D7: Bias in selection of the reported result.

Judgement
- Moderate
+ Low

FIGURE LEGENDS

FIGURE 1. Major components of transcranial focused ultrasound stimulation and its sonication paradigm in low-intensity use.

A: Illustration of the major components of the transcranial focused ultrasound stimulation of the brain, highlighting the usual cigar-shaped form of the acoustic intensity. B: Standardization of the main parameters used to describe the pulsed ultrasound stimulation.

FIGURE 2. Selection process for the studies included in this review.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) review flowchart for this systematic review.

FIGURE 3. Brain areas targeted in studies using transcranial focused ultrasound in humans.

Digital brain reconstruction of a healthy volunteer's magnetic resonance in 3D Slicer⁸⁰ using the FreeSurfer image analysis suite⁸¹ for segmentation according to the Desikan-Killiany atlas⁸², highlighting areas of interest.

FIGURE 4. Quality assessment of studies using the Risk Of Bias In Non-Randomised Studies (ROBINS-I).

Generated with the Robvis tool⁸³.

TABLE 1. Extracted data from studies that evaluated the effects of transcranial focused ultrasound sonication in humans.

Reference	N	M/F	R/L	Age	Sample	Brain area	Neuronavigation	Outcomes	Measurements	Summary of results	Safety
Legon W et. al., 2014 ⁷³	10	N/A	N/A	N/A	Healthy	Left S1	EEG electrode position (CP3)	SEP and sensory detection threshold changes when tFUS is applied -100 ms from MNS	EEG around tFUS transducer (C3, CP1, CP5, P3)	Reduction in mean amplitude of SEP recorded from C3 in tFUS group (short- and late-latency)	N/A
Mueller J et. al., 2014 ⁸²	18	11/7	18/0	18 – 54	Healthy	Left S1	EEG electrode position (CP3)	SEP changes when tFUS is applied -100 ms from MNS	EEG (CP5)	1) Change in β band of intrinsic brain activity. 2) Change in both β and γ bands in late SEP (total spectral power)	N/A
Lee W et. al., 2015 ⁴¹	12	8/4	N/A	21 – 43	Healthy	Bilateral S1	Fiducial markers (software not mentioned)	Sensory detection without external stimulation	1) Subjective perception 2) EEG	Response rate = 100%	Estimated temperature rise of 0.007°C
Ai L et. al., 2016 ⁴³	6	N/A	N/A	20 – 36	Neurologically healthy	M1 and S1 (dominant hemisphere)	Stereotactic neuronavigation system (Rogue Research Inc, CAN)	BOLD signal	fMRI	1) M1/S1 = 50% BOLD activity detection	Max 1°C increase (tFUS/MRI)
Lee W et. al., 2016a ⁷⁴	19	14/5	N/A	20 – 45	Healthy	V1	fMRI-based positioning (1cm tolerance from target)	Sensory detection without external stimulation	1) Subjective perception 2) EEG (PO7 or PO8; responsive subjects, N = 10)	1) Full responsiveness = 57.9% 2) Activation of V1 and areas of the visual pathway (fMRI) the 3) Modulation seen in EEG	1) No changes in mental or physical status or discomfort associated with the procedure 2) Follow-up anatomical MRI (up to

											3) fMRI (responsive subjects, N = 11)		4 weeks) did not identify any radiological abnormalities
Lee W et. al., 2016 ^{b85}	10	8/2	N/A	23 – 34	Neurologically healthy	Left S1 and S2	Custom-built MRI-guided system with helmet and fiducial trackers	Sensory detection without sensory perception after 20 tFUS stimulation events	1) Quantitative subjective perception 2) MRI/CT (calcification screening) 3) Clinical neurological assessment 4) mini-mental state examination	1) S1 = 68% (SD 28%) 2) S2 = 59% (SD 22%) 3) S1/S2 = 61% (SD 26%) 4) At least 1 response rate = 90% of participants	1) No adverse changes or discomforts across all subjects 2) No abnormal findings in neurological examination and mini-mental state examination		
Monti, 2016 ³⁹	1	1/0	N/A	25	Severe brain injury	Thalamus	MRI guidance	Recovery from minimally conscious state	1) Clinical assessments of levels of consciousness and responsiveness 2) Coma Recovery Scale-Revised ⁹⁸ 3) Timing: one day pre-sonication, on day of sonication (pre- and post-) and one day post-sonication	1) Coma Recovery Scale-Revised measurements = 15 (day prior), 14 (day of, pre), 13 (day of, post), 17 (day after) 2) Progressive clinical improvement (attempt to walk 5 days after sonication)	The procedure was "well tolerated and safe"		

Legon W et. al., 2018a ⁴²	50	16/34	47/3	19 – 38	Healthy	M1	Stereotactic neuronavigation system (Rogue Research Inc, CAN). Target tolerance $\leq 1\text{mm}$	1) Single pulse MEP recruitment (N = 12) 2) Paired-pulse MEP (N = 10) 3) Reaction time (N = 18)	EMG	1) MEP attenuation 2) tFUS attenuation of TMS-induced MEP 3) Reduction of muscle reaction time	1) No severe symptoms were reported 2) None of mild to moderate symptoms persisted after the stimulation
Legon W et. al., 2018b ⁴⁰	20	6/14	20/0	18 – 33	Neurologically healthy	Left somatosensory nuclei of thalamus	Stereotactic neuronavigation system (Rogue Research Inc, CAN)	1) SEP 2) Sensory detection thresholds	EEG electrode positions (five electrodes, near CZ)	1) Attenuation of EEG amplitude 2) Reduced detection threshold (comparison with chance)	N/A
Ai L et. al., 2018 ⁴⁴	5	3/2	4/1	20 – 25	Healthy	Contralateral dominant thumb representation (M1)	Stereotactic neuronavigation system (Rogue Research Inc, CAN)	Spatial restriction of tFUS neuromodulation	fMRI	Increase in BOLD activation volume in the thumb representation site of M1 without activation of subjacent areas	N/A
Sanguinetti JL et. al., 2020a ⁸¹	24	N/A	24/0	N/A	Healthy	rIFG (Brodmann Area 45)	F8 electrode position (EEG)	Mood enhancement	VAMS ^{99, 117}	1) Global Affect improved after 20 minutes 2) tFUS did not improve Global Vigor	1) No variation in heart-rate variability or respiratory sinus arrhythmia 2) Hearing from tFUS (N = 15)
Sanguinetti JL et. al., 2020b ⁸¹	9	(5/4)	9/0	19.2	Healthy	rIFG (Brodmann Area 45)	F8 electrode position (EEG)	1) Mood enhancement 2) fMRI (20 minutes after sonification)	1) VAMS 2) fMRI (20 minutes after sonification)	1) Improvement in both Global Affect and Global Vigor after 30 minutes	N/A

2) Alteration in brain activity

2) Increase in connectivity with premotor cortex
3) Decrease in connectivity with Broadmann Areas 11, 19, 25, 32, 35, 36, 37, 47

Badran BW et. al., 2020 ⁷⁸	17	N/A	N/A	N/A	Healthy	Right anterior third of thalamus	Stereotactic neuronavigation system (Rogue Research Inc, CAN)	Changes in thermal sensation, pain or tolerance after tFUS	Quantitative thresholding	sensory	tFUS significantly reduced thermal pain threshold after 20 min, but not thermal sensation or tolerance	Reported no adverse events
Julien L et. al., 2020 ⁹⁷	7	2/5	N/A	20 – 24	Unclear	Left S1	N/A	SEP changes with tFUS at -200, -100, and +500 (control) ms from median nerve stimulation	EEG (N20 and P27)		Dose-dependent difference in both N20 and P27, with -200ms tFUS group having greater magnitude of N20 and decreased magnitude of P27 when compared to control group	Reported no adverse events
Brinker ST et. al., 2020 ⁸⁰	1	0/1	N/A	26	Patients with TLE	Left hippocampus	MRI-guided open-source neuronavigation package	1) Description of laboratory-built device platform for tFUS neuromodulation in adults with drug-resistant TLE	Descriptive study		Description of successful multi-session therapeutic neuromodulation	N/A

2) Evaluation of
safety

Yu K et. al., 2021 ⁴⁵	15	10/5	N/A	33.39	Healthy		Left primary leg motor cortical region	Optical-based brain-navigation system (Localite GmbH, Germany) with helmet	Effect of PRF on EEG movement-related cortical potential source activity		Dose-dependent modulation of voluntary movement-related cortical activity by tFUS	Reported no adverse events
Stern JM et. al., 2021 ⁷⁷	8	3/5	N/A	22 – 65	Patients with TLE		Anterior temporal lobe	MRI guidance	Safety of tFUS	1) Post-surgical microscopy 2) Neuropsychological testing	No evidence of apoptosis or tissue damage	No evidence of apoptosis or tissue damage
Liu C et. al., 2021 ⁷⁹	9	5/4		35.77	Healthy		Left primary finger sensory cortical region (S1)	Optical-based brain-navigation system (Localite GmbH, Germany) with helmet	Correct responses when asked about the higher frequency of two vibrating stimuli	1) Percentage of correct responses 2) EEG responses 3) Brief survey for adverse effects	1) After tFUS, patients had a higher percentage of correct responses than sham 2) EEG: N30 and P100-N170 enhanced in tFUS group	Reported no adverse events
Kim YG et. al., 2022 ⁷⁵	8	3/5	8/0	45 – 65	Healthy		Bilateral Medial Prefrontal Cortex	Optical-based brain-navigation system	EEG changes in EEG response to different stimulation protocols		Different changes in EEG depending on stimulation protocol.	No subject reported any side effects

M/F: Male/female ratio; R/L: right-handed/left-handed ratio; EEG: electroencephalogram; MNS: median nerve stimulation; SEP: somatosensory evoked potentials; tFUS: transcranial focused ultrasound; N/A: not available; MRI: magnetic resonance imaging; fMRI:

functional magnetic resonance imaging; BOLD: blood oxygenation level dependent; CT: computerized tomography; SD: standard deviation; EMG: electromyography; MEP: motor evoked potentials; TMS: transcranial magnetic stimulation; rIFG: right inferior frontal gyrus; VAMS: Visual Analogue Mood Scale; TLE: temporal lobe epilepsy; US: ultrasound.

TABLE 2. Sonification parameters used by studies investigating the role of transcranial focused ultrasound in humans.

Reference	Af (kHz)	PD (ms)	PRP (ms)	PRF (kHz)	Duty cycle	SD (ms)	ISPPA (W/cm ²)	ISPTA (W/cm ²)	MI	S/I
Legon W et. al., 2014 ⁷³	500	0.36	?	1	?	500	0.12 – 50	?	?	I
Mueller J et. al., 2014 ⁸²	500	0.36	?	1	?	500	23.87	?	1.13	?
Lee W et. al., 2015 ⁴¹	250	1	2	0.5	50%	300	0.7 ± 0.5	0.35	0.62	S
Ai L et. al., 2016a ⁴³	500	?	?	?	36%	500	6	?	?	?
Ai L et. al., 2016b ⁴³	850	?	?	?	50%	500	6	?	?	?
Lee W et. al., 2016a ⁸⁵	210	1	2	0.5	50%	500	35	17.5	?	S
Lee W et. al., 2016b ⁷⁴	270	1	2	0.5	50%	300	16.6	1.5 ± 0.9	0.2 – 1.2	S
Monti, 2016 ³⁹	650	0.5	?	0.1	?	?	?	720	?	S
Legon W et. al., 2018a ⁴⁰	500	0.5	1	1	36%	500	7.03	?	0.56	I
Legon W et. al., 2018b ⁴²	500	0.5	?	?	?	?	17.12	6.16	0.9	I
Ai L et. al., 2018 ⁴⁴	500	0.5	1	1	36%	500	16.95	?	0.97	S
Sanguinetti JL et. al., 2020 ⁸¹	500	0.065	23	0.04	0%	30000	54	0.13	1.79	I
Badran BW et. al., 2020 ⁷⁸	650	5	720	0.01	5%	30000	?	0.719	?	I
Julien L et. al., 2020 ⁹⁷	350	0.5	0.542 (median)	1	100%	500	9.4 (median)	3.2 (median)	0.95 (median)	?
Brinker ST et. al., 2020 ⁸⁰	548	0.5	?	0.5	36% – 50%	500	?	0.5 – 2.25	?	?
Yu K et. al., 2021 ⁴⁵	500	0.002	0.003 and 0.0003	0.3 and 3	6% and 66%	500	1.17	0.702	?	S
Stern JM et. al., 2021 ⁷⁷	650	0.5 and 2	4 and 10	0.1 and 0.25	5% and 50%	0.5 and 30	0.66 – 152.1	0.72	0.14 – 2.14	?
Liu C et. al., 2021 ⁷⁹	500	0.002	0.003	0.3	6%	0.5	1.1	67.13	?	S
Kim YG et. al., 2022 ⁷⁵	250	0.5	?	?	5% and 70%	300			0.30	?

Af: Acoustic Frequency; PD: Pulse Duration; PRP: Pulse Repetition Period; PRF: Pulse Repetition Frequency; SD: Stimulus Duration (considering intervals between pulses); I_{SPPA} : Spatial peak pulse average intensity; I_{SPTA} : Spatial peak temporal average intensity; MI: Mechanical Index; S/I: outcome of the study, being registered as stimulation (S) or inhibition (I).

5.2. Open-source neuronavigation method

As noted in the previous section's discussion, neuronavigation plays a significant role in tFUS stimulation in humans. This is mainly because, differently from TMS, a minor tilt or movement in the transducer can shift the deep focus of sonication to a completely different brain structure. In the past, multiple trials were made with the transducer inside of the MRI machine to align it to the focus and guide neuromodulation. Still, it is unpractical in the clinical setting.

For this reason, an open-source method with infrared camera is proposed. The open-source model allows multiple researchers to contribute to possible optimizations. Furthermore, it decreases research costs, encourages tFUS research, and enable more clinical trials. To the best of the author's knowledge, there are few open-source methods for tFUS stimulation in humans to date. A method described by Preiswerk et. al.⁸⁶ was adopted to be studied due to its basis on open-source software. Although it was not peer-reviewed, it proved to be adequate to the available material and, therefore, was optimized in collaboration with the original authors.

**OPTIMIZED OPEN-SOURCE NEURONAVIGATION METHOD FOR
TRANSCRANIAL FOCUSED ULTRASOUND BRAIN STIMULATION IN HUMANS**

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1 **ABSTRACT**

2 Neuromodulation using low-intensity transcranial focused ultrasound is an
3 upgrowing field of study. Although it has shown potential due to its high spatial
4 specificity and capacity to reach deep brain structures, those characteristics also
5 demand specific software and hardware for neuronavigation.

6 This paper presents an open-source method of neuronavigation refined through
7 its use in multiple clinical trials after years of use and refinement in multiple centers.

8 The objectives were to address key limitations of open-source resources for
9 neuronavigation, to integrate multiple available tools and to improve the user interface
10 for practical use in research and clinical settings. The method was tested on several
11 subjects and its results are reliable, with no aberrant variations across different
12 settings. The user manual is open to use, and its purpose is to increase feasibility and
13 availability of ultrasonic sonification for neuromodulation studies.

14 **Keywords:** neuronavigation, transcranial focused ultrasound, humans, brain
15 stimulation

1 INTRODUCTION

2 The use of low-intensity transcranial focused ultrasound (tFUS) for
3 neuromodulation has become increasingly studied over the past decade, mainly due
4 to its non-invasive profile and high spatial specificity³⁸. Nowadays, neuronavigation is
5 considered indispensable for tFUS stimulation in humans to ensure the correct
6 positioning of the transducer³. It requires at least one structural magnetic resonance
7 image (MRI) sequence and an optical-based camera to track real-time movements⁸⁰,
8 ⁸⁵. Studies based on a trial-and-error method were tried in the past^{39, 74}, but they
9 required at least three MRIs to guide a single sonication³⁹. This method may suit the
10 research environment but be unsustainable in clinical practice.

11 To the best of our knowledge, only one open-source neuronavigation method
12 designed explicitly for tFUS stimulation in humans is freely available⁸⁶. Its core
13 component is 3D Slicer, an open-source software that enables the creation, edition,
14 and visualization of three-dimensional models using anatomical coordinates¹¹⁸. This
15 method is currently used in three clinical trials, but it lacked peer revision. Its practical
16 use is challenging due to its non-user-friendly settings and meticulous implementation
17 steps. Furthermore, its results are poorly stable, visually limited, and ignore crucial
18 evidence such as skull deviation of the ultrasonic waves⁷⁶. In addition, it requires
19 refined neuroanatomy expertise to target deep brain structures.

20 This study aims to analyze the limitations of freely available neuronavigation
21 methods for tFUS brain stimulation and establish a novel methodology for open-source
22 neuronavigation.

24 MATERIALS AND METHODS

25 This optimized open-source neuronavigation method was developed by
26 Preiswerk et. al.⁸⁶ and further improved by the authors of this manuscript. Therefore, it
27 was possible to keep its primary structure and improve usability. The core software
28 employed in this study are: (1) 3D Slicer version 5.4.2¹¹⁸, (2) FreeSurfer suite version
29 7.3.2¹¹⁹, and (3) Plus toolkit version 2.8.0¹²⁰. The hardware was composed of (1) a
30 Windows 10 computer with 16GB-RAM and a 2.80 GHz processor, (2) a Macbook Air
31 M2 with 8GB-RAM (3) an NDI© Polaris Vicra stereotactic infrared camera (Ontario,
32 Canada), and (4) a set of 3D-printed devices with infrared-reflective spheres. The

interaction between software and hardware used in this method is illustrated in Figure 1A.

The points of interest were elected according to their relevance by users of the previous method. They are: (1) intuitiveness and learning curve of the method, (2) matching the calculated point of maximum acoustic intensity (hereby named “focus”) with the area of interest in a digital three-dimensional space using a stereotactic camera, (3) capacity to guide multiple sonications in a single patient over different sonications, keeping the same stimulation region over time, (4) reduction of the high neuroanatomy expertise to define the targeted point, (5) optimization of the number of transducers per scene, and (6) alignment of possible acoustic pressure map aberration by the skull.

RESULTS

The original files for neuronavigation can be accessed through <https://github.com/ctmmufmg/CTMM-UFMG>. 3D Slicer was kept as the main software for this method, and the instructions were rewritten to improve the method’s learning curve and allow the real-time tracking of multiple transducers. As the software allows multiple pre-defined scenes, transforms, and three-dimensional models, a universal file containing the primary data of the neuronavigation structure is available in a GitHub folder. It can be read entirely using 3D Slicer version 5.4.2. Keeping the original transform hierarchy is strongly recommended during the entire neuromodulation process.

Neuronavigation method description

This method intends to show the real-time position of the point of maximum acoustic pressure generated by the tFUS transducer. A three-dimensional head is digitally reconstructed based on the patient’s MRI, and the tFUS transducer is illustrated by a cylinder in 3D Slicer’s three-dimensional space, moving according to its distance from the patient. The required precision for neuronavigation is only possible if the fiducial objects are firmly attached to the patient’s head and the transducer.

Each “fiducial object” comprises at least three non-linear reflective spheres. The optical camera emits and captures infrared signals, allowing real-time tracking of pre-registered fiducial objects. Their patterns compose the basis of the digital

reconstruction of both the patient's head and the transducer. A third fiducial object (usually a stylus) is needed to register specific points in actual structures, which will be translated into digital models. Examples of fiducials objects are depicted in Figure 1C. As multiple pre-configured models, transforms, and shapes are needed for this neuromodulation method, all the files necessary are hosted in a specific GitHub folder. The user's manual can be found in Supplementary File 1.

Target definition

The previously available neuronavigation scene allows the selection of a brain point that the tFUS focus must target in real-time, requiring millimetrically impractical precision. The current method allows the expansion of the target into a fine-shaped volume, which is possible due to FreeSurfer processing. Besides, the focus is also changed from a single point in space to a sphere of a 2mm radius. This value is empirical, based on previous data on the focus' distortion by the skull¹⁰⁶. Therefore, the aberrational behavior of the tFUS beam would, theoretically, respect a precise margin of error and would be covered by this new method.

Translating the point-based match into a volume-based method is fundamental to increase user-friendliness and practicability. Matching two points in a digitally built environment is impractical due to the intrinsic margin of error of any stereotactic camera. FreeSurfer processing eliminates the need of precise neuroanatomical knowledge and provides a whole volume that can be targeted. Turning the focus into a sphere allows researchers to establish a "margin of error" distance that corresponds to the hypothetical aberrance that the skull causes on the acoustic pressure map. By definition, if the sphere is completely inside the volumetric structure targeted, any aberration equal or less than the radius of the sphere would not cause misrepresentation of the focus.

Use of multiple transducers

The current method allows the insertion of multiple tFUS transducers since there are already published studies⁸⁵ and ongoing clinical trials using 3D Slicer with more than one transducer. For this, each transducer must be matched to a different spatial configuration of reflective spheres, as illustrated in Figure 1C. This allows the camera to distinguish each object and translate their input into the 3D Slicer's scene.

Treatment planning

The tFUS's cigar-shaped pressure map was well-established in several previous studies^{73, 75, 82, 97}. As mentioned before, the maximum acoustic pressure point is recognized as a “focus” with a fixed distance from the transducer's surface, although the stimulation map must consider the whole cigar-shaped volume. One of this neuronavigation method's aims is to minimize off-target effects, allowing a spatial plan before the sonication occurs. For doing so, a line is traced between the targeted deep structure and a point in the scalp, allowing a precise, fast real-time transducer positioning.

Multiple time-spaced sonications

The tFUS's cigar-shaped pressure map (depicted in Figure 2A) is not completely symmetrical as a sphere, and, consequently, configures a two-dimensional axis. Although the previous point-based method may be reliable for single sonications, multiple sessions require repetitive alignment of the tFUS axis, therefore demanding a second point in the three-dimensional scene. This alignment can be achieved by establishing two fixed points in space (denominated “deep target” and “superficial target” in the Supplementary File 2).

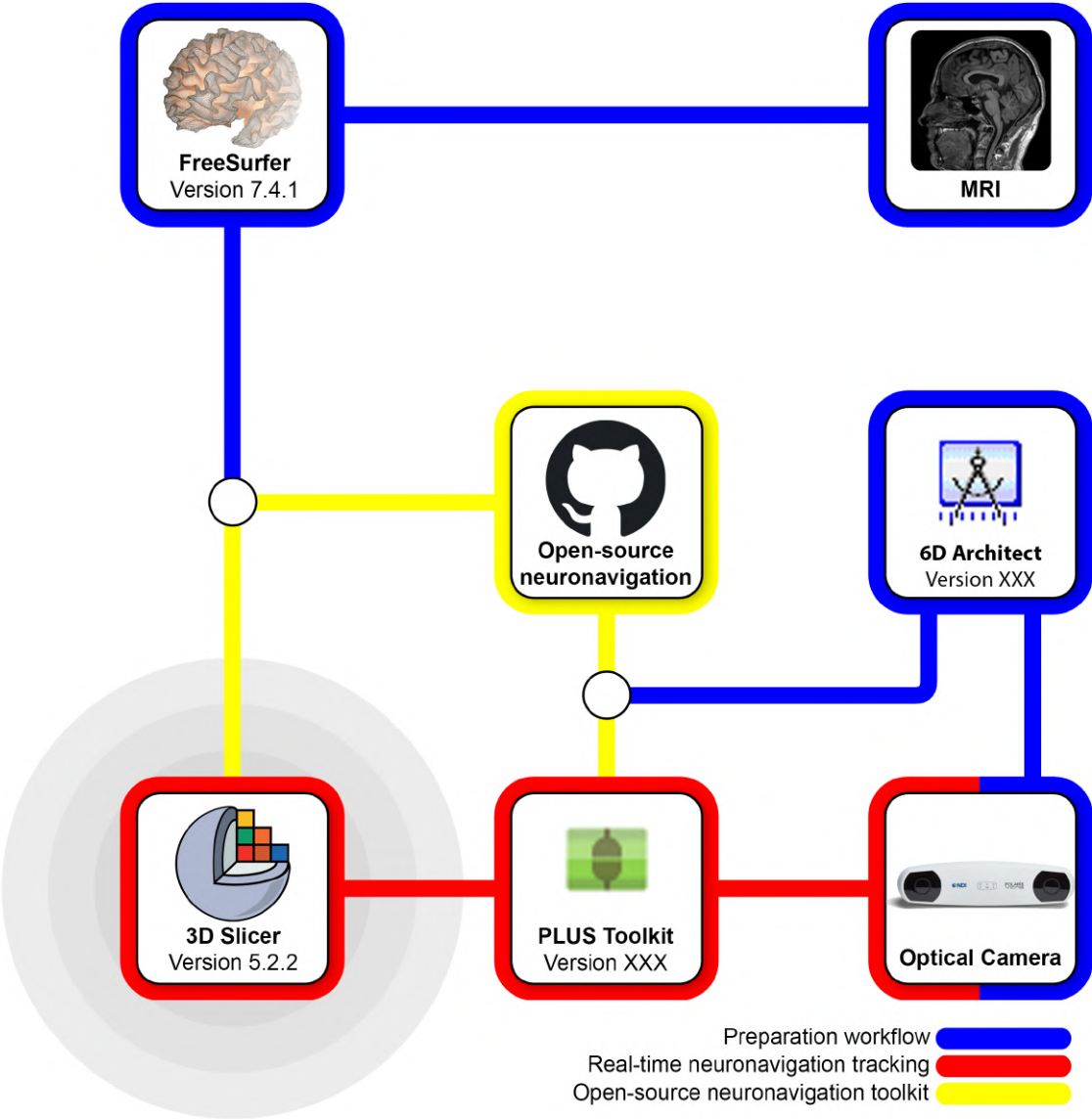
3D visualization

While the previous method relied upon the approximation of the focus to the targeted point in three different 2D slices, this new approach is based on five different windows: three of them are preserved from the initial method, centered at the focus in the axial, coronal and sagittal planes, while two additional three-dimensional windows are added. The first is centered on the patient's head digital reconstruction, allowing quick scrutinization of the registration procedure. The second is a real-time view of the transducer's plane, which centers the target and approximates the transducer to the planned sonication line. An example of real-time neuronavigation is provided in Figure 2B.

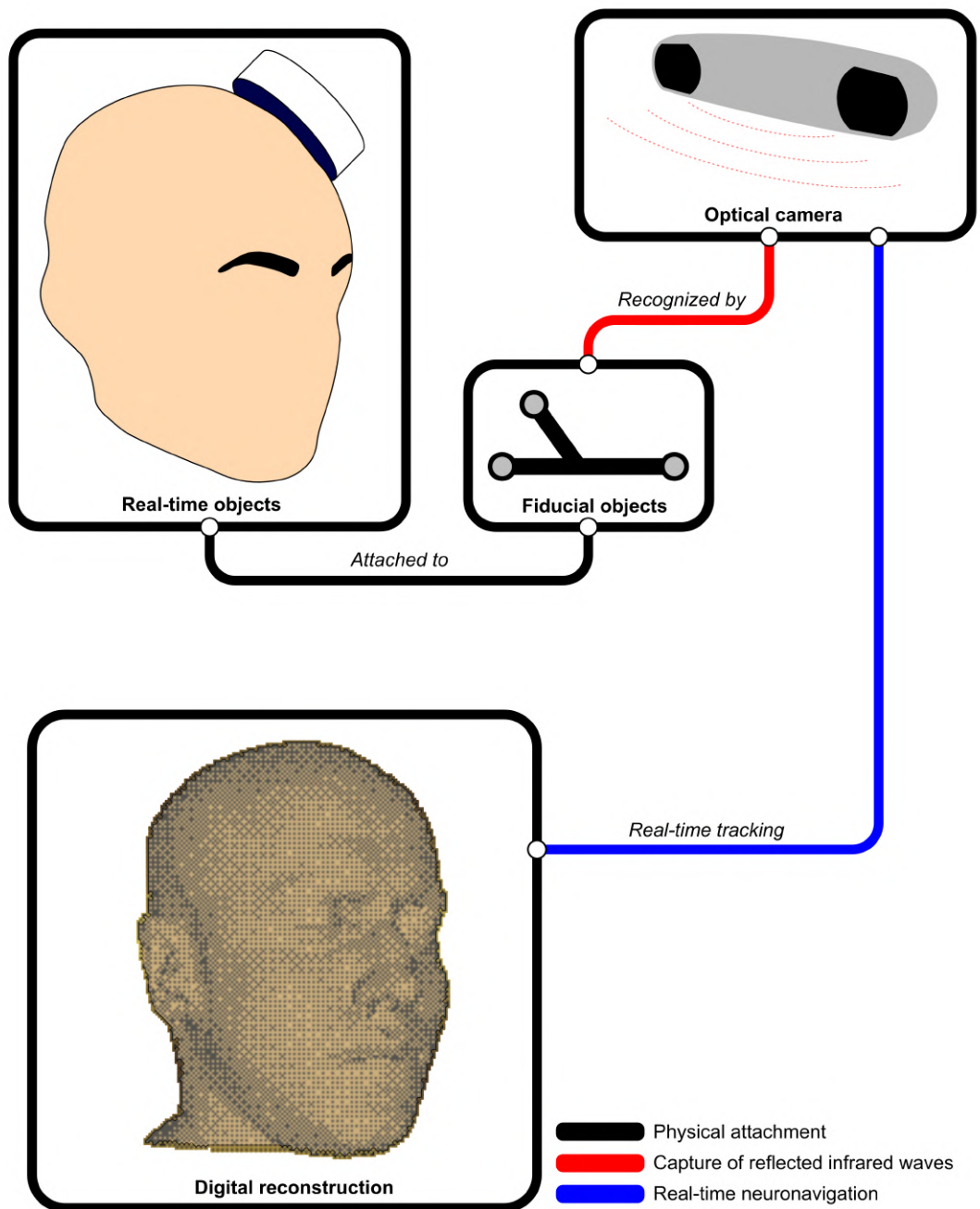
CONCLUSIONS AND OUTLOOK

1 To the best of our knowledge, this is the first open-source method submitted for
2 peer review. Although it is based on a previously unpublished version⁸⁶, several
3 limitations were addressed to make it flexible and practical in multiple research
4 settings. In such a growing field as tFUS research, this open-source neuronavigation
5 method is expected to lower research costs and improve the democratization and
6 spread of further pre-clinical and clinical studies.

1 **FIGURE 1A**



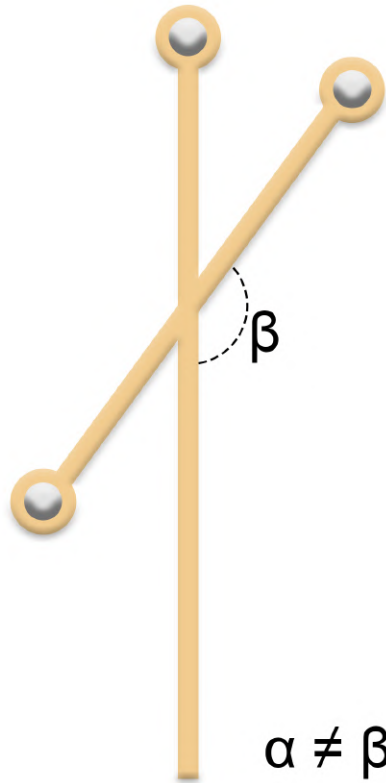
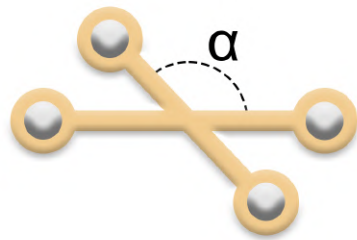
2
3

1 **FIGURE 1B**

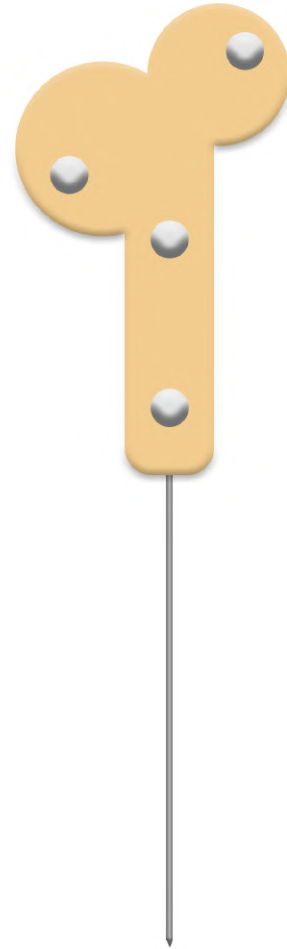
2

3

1 FIGURE 1C



$$\alpha \neq \beta$$



2

3

5.3. Feasibility of computationally generated 10-20 International Positioning to guide brain stimulation

The optimization of the neuronavigation method was a considerable step in proposing the clinical trial. However, there was still an aim to optimize the reproducibility and time needed to target neuromodulation specific areas, i.e., the prefrontal dorsolateral cortex (DLPFC). This brain region is related to the anterior parts of the medial and superior frontal gyri. If a reliable and automated method was used, repeated sonications over a single cortical area in different sessions would be faster and more reliable.

This concern led to efforts towards a feasible method to determine a reliable position for the transducer before sonication. Generally, the definition of the target is made by measuring the patient's head during the first session. In the International 10-20 system, the F3 represents the left DLPFC and F4 represents the right. These are the major brain areas to be neuromodulated in depression. The most used method to date to calculate these points is the BeamF3 method. It is a computerized method based on the measurement of the circumference, tragus to tragus and nasion toinion distances in centimeters. The method generates two numbers of distance in cm to be marked on the patient's head. The first is the distance along circumference from midline over the eyebrows towards the left ear. The second number is the distance from the vertex towards the first point. Generally, these points are marked on a cap placed on the patient's head. While this strategy helps to decrease the time need to find the target for each session, these caps can slip and be suitable to stretching. For TMS, variations in half a centimeter may not be so problematic. On the other hand, these variations are problematic considering the significant smaller area of stimulation generated by the tFUS transducer.

Therefore, the use of a cap is not recommended and headgears with stereotactical markers are generally used. On the other hand, these devices still need coordinates for the neuronavigation software. In order to optimize the time with the patient for neurostimulation, pre-processing of the MRI and the use of integrated 10-20 systems into the neuronavigation software. If reliable and accurate to underling cortex, this integration can lead to improvement in treatment planning before stimulation sessions.

1 To better explore this characteristics, this study aims to test a computerized
2 method for finding the F3 point with a single morphological MRI, its accuracy to
3 underlying cortex, optimizing patient's time and allowing for a three-dimensional
4 preparation before the actual sonication.

5 A succinct abstract of preliminary data of this study was approved as an abstract
6 for the Organization of Human Brain Mapping 2024 meeting (South Korea) with the
7 number 2135.

8

COMPUTATIONAL CORRELATION BETWEEN THE INTERNATIONAL POSITIONING AND UNDERLYING CORTICAL AREAS

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None.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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1 INTRODUCTION

2 The International 10-20 system was introduced in 1958 to standardize the
3 positioning and nomenclature of electroencephalogram (EEG) electrodes in
4 humans¹²¹. It has been widely used as a diagnostic tool for epilepsy, management of
5 coma, and surgery planning¹²². It has also been proposed that EEG can play a major
6 role in the near future in sleep medicine and diagnosing other neuropsychiatric
7 conditions^{122, 123}. Currently, the International 10-20 system is considered the best way
8 to non-invasively assess the underlying cortical area of a given scalp point¹²⁴.

9 This historical characteristic of the International 10-20 system made it especially
10 interesting for guiding non-invasive neuromodulation¹²⁵. Since the International 10-20
11 system classically correlates to the brain cortex, it is especially interesting for guiding
12 techniques that aim for cortical areas, such as transcranial magnetic stimulation (TMS).
13 Nevertheless, even clinical trials investigating potentially deep-brain reaching
14 techniques, such as transcranial direct current stimulation¹²⁶, use the 10-20 system for
15 guidance. Herwig et. al. (2003) proposed that five of the nineteen points were of
16 considerable importance for such guidance: F3, F4, T3, P3, and the point in the middle
17 of the line traced between T3 and P3, hereby named TP3. Since the standard
18 terminology of the International 10-20 system infers that odd and even numbers refer
19 respectively to the left and right sides, the laterality will be assumed from now on.

20 Several studies consider the correlation between F3 and F4 with the
21 dorsolateral prefrontal cortex (DLPFC) as a premise¹²⁷⁻¹³¹. The DLPFC is considered
22 to be related with “cognitive” and “executive” functions⁵⁹, impaired in many
23 neuropsychological conditions. Probably the most well-known use of F3 corresponding
24 to the DLPFC is in the treatment of refractory depressive disorder, for which TMS has
25 been approved⁵⁹. Consensually, the DLPFC seems to be commonly correlated with
26 the neuroanatomy of depression across multiple psychoradiological studies¹³².
27 Although the classical protocol of TMS stimulation focuses only on F3, protocols that
28 apply low-frequency TMS modulation over the right DLPFC have also been
29 proposed⁵⁸, setting F3 and F4 as probably the most well-studied scalp points for TMS
30 stimulation.

31 Similarly, the P3 was stimulated in a considerable number of studies, which
32 related it to visuospatial processing^{133, 134}, hazard rating¹³⁵, and complex motor-
33 executive processes¹³⁶. Although studies guided by the P3 point are heterogeneous,

1 they agree that this point corresponds to the parietal cortex, which is complex: it seems
 2 to have an essential role in visually-guided reach planning¹³⁷, a hypothesis that
 3 congregates many of the abovementioned studies but involves multiple functions.

4 Although T3 itself is not a common target of TMS interventions, its definition is
 5 essential to define the TP3 point. A recent meta-analysis found eleven robust sham-
 6 controlled studies that guided a TMS intervention exclusively on the TP3 in the
 7 treatment of auditory hallucinations in schizophrenia¹³⁸. The studies consider TP3 to
 8 be naturally correlated to the temporoparietal sulcus, an area related to face-emotion
 9 processing, attention, theory of mind operations, and memory¹³⁹. It has been proposed
 10 as a prefrontal-parietal hub important for cognitive processes¹⁴⁰ or socially related
 11 relational metrics¹⁴¹, both important for the physiopathology of schizophrenia.
 12 Interestingly, one of the first case series that mentions the TMS inhibition over TP3 to
 13 treat auditory hallucinations¹⁴² seems to be motivated by an image-guided study that
 14 found hyperactivation of the temporoparietal cortex in five patients with active auditory
 15 hallucinations¹⁴³. It suggests that the TP3 guidance follows an empirical succession of
 16 studies, which were proven by the abovementioned meta-analysis to have indeed the
 17 potential to treat auditory hallucinations.

18 Even though the International 10-20 system has been extensively used as a
 19 superficial correspondent to an underlying cortical atlas of the brain, few studies
 20 attempted to prove this correlation. A historical study by Homan et. al. (1987) used
 21 computerized tomography (CT) to establish such correlation, but the methodology was
 22 relevantly limited due to the chosen image method¹⁴⁴. Similarly, another historical study
 23 tried to use CT to demonstrate this correlation in the occipital cortex¹⁴⁵. More recent
 24 studies used the International 10-20 system to approximate a superficial brain atlas,
 25 focusing on the standard deviation of different measurements using the system or
 26 proposing new automated projections based on the system^{124, 146}. All of the
 27 abovementioned studies deserve their respective credit. Still, the number of
 28 participants was limited, and none of them focused on answering the main question
 29 that arises when guiding cortical stimulation using the International 10-20 system:
 30 when using anatomically-guided, clinically-viable positioning via the International 10-
 31 20 system, is the applicator reaching the desired cortical region?

32 This study aims to analyze if a computational, reproducible method of
 33 distributing the International 10-20 system points is consistent with the subjacent

cortex in a given MRI. Besides, it also intends to analyze the relative distance of the most relevant points to the nearest cortices, providing insight into possible tendencies of mistake and, in the case of the DLPFC, how approximated are F3 and F4 to the functional area described previously.

MATERIALS AND METHODS

This article is part of a larger study ($n > 190$) performed at the Federal University of Minas Gerais (UFMG), which required an MRI for each participant. UFMG's Institutional Review Board approved the study under the number CAAE E3768820.1.0000.5149. A written consent form was signed by all participants, including rights to image processing and analysis. The original research was focused on patients diagnosed with Coronavirus Disease 2019 (COVID-19). Therefore, the pooled sample comprised patients aged between 18 and 60 years, with confirmed diagnosis of COVID-19 via real-time polymerase chain reaction. The eligibility criteria, recruitment, and neuroimage used in the larger study are detailed in Supplementary File 1. The current research randomly chose 64 MRI from the largest study due to convenience. No additional inclusion or exclusion criteria were added.

Hardware and software

Every procedure described in this study was performed on a Mac Pro M2 2019, 12-core GPU. Every software is open-source to improve reproducibility and research access. XQuartz version 2.8.5 was required. FreeSurfer version 7.3.1 was used for cortical parcellation based on the Desikan-Killiany Atlas (DKA). 3D Slicer version 4.2.2 was used to integrate multiple extensions, digitally reconstruct the participant's head, and collect data. 3D Slicer's extensions used in this study were (1) SlicerFreeSurfer, (2) Geodesic Slicer, and (3) Fiducials to Model Distance.

Computational procedure and data collection

After inclusion, the MRIs were processed using FreeSurfer and imported into 3D Slicer via the SlicerFreeSurfer extension. This loaded a T1 Magnetic-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence of images and multiple segments corresponding to the DKA segmentation. A "segment", or a "model", is read by 3D Slicer as a well-defined three-dimensional shape. The process included 103

segments into each scene, composed of 62 (31 for each side) cortical segments and 41 deep structures. The deep structures were deleted and the cortical segments were copied into three-dimensional models.

The next step consisted of reconstructing the participant's head in the three-dimensional space of 3D Slicer and determining pre-established anatomical fiducials (nasion, inion, left pre-auricular, and right pre-auricular). A "fiducial", or a "marker", is read by 3D Slicer as a point in space with unique coordinates. An experienced psychiatrist trained three different evaluators to determine the anatomical fiducial points. The nasion and pre-auricular markers were placed over visual inspection.

The inion placement was more challenging due to the intrinsic lack of tactile feedback applicable to clinical 10-20 International Setting electrode placement. First, the image was rotated around the interior-superior axis, to align the left and right hemispheres symmetrically. This was needed to provide a single sagittal slice view that corresponded to the median line with maximum accuracy. Once it was performed, an already published method for placing the inion using MRI was performed: a line was traced between the anterior commissure and the posterior commissure, and a second line was traced, parallel to the first one, passing through the nasion point. The point on which this second line crossed the skin was determined as the inion.

Once the four fiducial points were established, Geodesic Slicer extension was used to calculate the position of the 10-20 International Setting points and set their coordinates as 19 markups. In summary, it does that by creating a mesh that corresponds to the patient's head and calculating the distances in its surface as defined by Jaspen et. al. As only 5 markups were of interest to this study, 18 markups (including the anatomical fiducial points) were excluded.

After that, the minimum distance between each markup and each of the 104 models was calculated using the Fiducial to Model Distance extension. In brief, it does so by finding the point in the model's surface with the minimum distance to the markup and displaying it in a table. The modules of these values were collected for each model-markup combination, and the three lowest values were collected. For every patient, there was no divergence among the three cortical models closest to each markup. If a markup was not equidistant to two different cortices, the closest cortex was labeled as the subjacent.

Inter-evaluator assessment

The study was performed by three independent researchers, each blinded to the other's partial results. As a large part of the method is visual (e.g., surface anatomical markups placement, definition of anterior and posterior commissures), reproducibility is highly dependent on inter-evaluator non discrepancy. Data was initially collected quantitatively (the distance of each markup to the three nearest cortices), and then transformed into qualitative information (subjacent cortex). This way, a contingency table was created showing the number of results for each subjacent cortex of each markup, for each evaluator.

Statistical analysis

Three major analyses were performed: (1) quantitative differences between each cortical-point distance for each individual; (2) qualitative differences between the frequencies of each cortical-point subjacency; and (3) inter-evaluator assessment. The first analysis is important to critically analyze the results, given the anatomical individualities of each cortical area that is intended to be stimulated by the 10-20 system guidance. Each MRI provided 60 distances between specific points and cortices. The Kolmogorov-Smirnov test was used to test the normality of these distances. The difference between each point and its three nearest cortices was evaluated using the Analysis of Variance test (ANOVA). Post-hoc Tukey Honestly Significant Difference was performed due to the intrinsic multi-evaluator setting of the study. Chi-square was used for both qualitative and inter-evaluator contingency table analysis. An alpha-value of 0.05 was set. Descriptive statistics were displayed as mean (standard deviation) or median (1st quartile – 3rd quartile). The Statistical Package for the Social Sciences Software (IBM SPSS Statistics, USA) version 22.0 was used for data analysis.

Classic two-dimensional cartesian graphics are not suited to display data composed of three components. Therefore, a graph based on the Gibbs Triangle illustrated the relative distances collected from each individual. The plot was designed using the TernaryPlot platform (Blom J, <https://github.com/JulesBlm/>). More explanation of how the Gibbs triangle was used to plot data is available in Supplementary File 1.

1 RESULTS

2 Of the 64 included MRIs, 4 (6.25%) could not be processed by FreeSurfer due
3 to unknown processing errors and were not analyzed. The sample comprised
4 individuals aged from 21 to 65 years old, mostly right-handed, who studied from 9 to
5 20 years. Age was normally distributed, while education's distribution was left-skewed
6 (skewness -0.53344, $p < 0.001$). The demographics are detailed in Table 1.

7 Statistical significance was found among all nearest cortices for F3, F4, T3, and
8 P3, as shown in Figure 1B and Table 2. In 46 (76.66%) cases, the left Rostral Middle
9 Frontal Cortex was subjacent to F3. In 51 (85%) cases, the right Rostral Middle Frontal
10 Cortex was subjacent to F4. In 38 (63.33%) cases, the left Medial Temporal Cortex
11 was subjacent to T3. In 46 (76.66%) cases, the left Inferior Parietal Cortex was
12 subjacent to P3. Differently, TP3 showed no statistical difference between the point's
13 distances to each of the three nearest cortices, although this triad has not changed
14 inter-individually. In the F3 analysis, one individual's point was equidistant to the left
15 Rostral Middle Frontal and the Superior Frontal cortices. In both the TP3 and T3
16 analyses, two individuals' points were equidistant to the left Middle Temporal and
17 Superior Temporal cortices. No equidistance was found in the F4 and P3 analyses. No
18 measurement placed the F3 or F4 adjacent to the Caudal Middle Frontal Cortex (left
19 and right, respectively). Similarly, no measurement placed the P3 adjacent to the left
20 Supramarginal Cortex.

21 The ANOVA analysis showed statistical significance among all cortices for F3,
22 F4, T3, and P3. The ANOVA analysis showed that the distances between TP3 and its
23 nearest cortices did not differ statistically. The distances' distribution according to each
24 10-20 markup are illustrated in Figure 2, and the ANOVA post-hoc analysis is detailed
25 in Table 2. Figure 3 shows the Ternary Plot for each 10-20 markup, highlighting a critical
26 area (displayed in yellow) inside of which the three cortices are almost equidistant from
27 the respective point (each distance contributes to at least 30% of the relative total
28 distance). No point in the F3, F4, or T3 plots fell into this critical area. One point (1.66%)
29 of the P3 analysis was plotted inside of it. On the TP3 analysis, 9 (15%) points were
30 plotted inside the critical area and each evaluator's means. Additionally, the F3 and F4
31 plots contained a red area in which a point would have a significant ($<30\%$) relative
32 proximity to the Caudal Middle Frontal Cortex. No points fell into this area.

The inter-evaluator analysis showed no statistical significance in any of the 20-10 international system points, as demonstrated in Table 3. This result was also illustrated in Figure 3 by visually examining the proximity of the mean relative distances measured by each one (marked as blue, yellow, and red triangles).

DISCUSSION

The International 10-20 system was introduced almost 80 years ago, and its markers' underlying cortical areas are considered prescriptive. However, to the best of the authors' understanding, no study has used a reproducible method to evaluate whether such markers represent the underlying cortex or region of interest. One of the most studied methods for cortical stimulation is TMS, which reaches a considerably large area and may be guided by the International 10-20 system.

Some authors consider that TMS guidance using the International 10-20 system does not consider inter- and intraindividual variability of cortical anatomy¹⁴⁷. They propose a new method of guidance called image-guided neuronavigation, which uses morphological images and specific software to guide the stimulation. According to Lefaucheur J.-P. (2010), a neuronavigation system must fulfill four essential prerequisites: (1) determining the cortical location of a given TMS target; (2) ensuring the reproducibility of TMS targeting during repeated sessions and/or in follow-up studies; (3) improving the accuracy of TMS motor mapping methods; and (4) determining the functional involvement of a cortical region¹⁴⁸. In addition, stereotaxic neuronavigation allows the use of robotic arms in repetitive sessions to improve the reproducibility and accuracy of such sessions¹⁴⁹.

In clinical therapeutical practice, perhaps the F3 point is the most significant marker of the International 10-20 system. This is justified by the approval of TMS for treating refractory depression⁵⁹, which frequently uses this point for guidance via the Beam F3 method⁶¹. However, a study has already pointed out that different methods for finding the TMS hotspot are considerably different⁶³, although the Beam F3 was concluded to present greater inter-applicator precision. This recent study illustrates how scalp markers are still of greater relevance in clinical practice due to their low cost and quick learning curve, in contrast with neuronavigation-guided interventions. On the other hand, TMS results are considered only modest¹⁵⁰, and their results seem to be optimized with neuronavigation when compared to standard techniques¹⁵¹. Studies

1 that compared functional image-guided neuronavigation proved it to be even more
 2 efficacious than morphological neuronavigation, and both perform better than simply
 3 targeting the P4 point in cognitive studies¹⁵².

4 The present study demonstrates that a computational model helps find the F3
 5 and F4, which statistically corresponded to the morphological area correlated with the
 6 DLPFC in all patients. This may contribute to future research and clinical applications
 7 of TMS and other cortical stimulation techniques that aim the DLPFC, either for
 8 depression or other neuropsychiatric conditions. This discovery is aligned with the
 9 previously mentioned studies, that points out that image-guided neuronavigation may
 10 increase TMS efficacy for depression¹⁵¹. This computational method is cost-effective
 11 since it is based on open-source software. It may be considered an image-guided
 12 neuronavigation that mixes morphological brain areas with previously established
 13 International 10-20 points. Besides F3 and F4, it also predicted with a high level of
 14 precision that the points T3 and P3 correspond to underlying temporal and parietal
 15 cortices, respectively. Besides, the sample of this study is highly correlated with the
 16 population in general, especially considering hand dominance.

17 The negative result shown for the TP3 markup can explain the high
 18 heterogeneity of studies in the field. Interestingly, the recent meta-analysis that
 19 evaluated the efficacy of TMS for auditory hallucinations found a statistically significant
 20 improvement in the pooled analysis¹³⁸. Still, most of the included studies failed to
 21 demonstrate the effectiveness of repetitive TMS against sham, and all studies used
 22 the TP3 point to guide the TMS intervention. Sommer et. al. (2018) have already
 23 proposed that neuronavigation may help guiding repetitive TMS for the treatment of
 24 auditory verbal hallucinations¹⁵³, but the state-of-the-art of such guidance seems to be
 25 modest to date.

26 Prudence is advised when analyzing the current study's results. The fact that
 27 TP3 is the point between T3 and P3 may falsely lead to the impression that the correct
 28 placement of both points will be sufficient to place the TP3 accurately. As the
 29 introduction mentions, the TP3 aims at the temporoparietal cortex, more importantly
 30 the Sylvian fissure¹⁴³. The analyses performed in this study focus on a group of
 31 patients, showing how the result of TP3 is heterogeneous even if both T3 and P3 are
 32 correctly correlated with the intended corresponding cortices. This is probably
 33 explained by inter- and intra-personal anatomical differences, but, more importantly,

the area aimed by TP3 is much more specific than the ones aimed by T3 and P3 separately. This result suggests that image-guided stereotaxic neuronavigation may be even more important for studies that aim at reducing auditive hallucinations than, for example, in studies that focus on the DLPFC.

CONCLUSION

The International 10-20 system is considered the gold-standard method for cortical neuroanatomy correlation with scalp markers. It has been used to guide cortical stimulation by many methods, mainly TMS. Despite its reputation as a reliable scalp marker for underlying cortices, few studies have validated such a correlation. This study provides a replicable, computer-based method for analyzing the association of different points of interest and underlying cortices with a relatively large number of subjects. In this study, the computational placement of F3, F4, T3, and P3 were consistent with underlying cortices. The falling of F3 and F4 over the Rostral Middle Frontal Cortex is highlighted since it has been previously considered an anatomical correspondent to the functional area known as the DLPFC. Even though TP3 has been used as a topographical marker for studies that aim at reducing auditory hallucinations, it did not correspond to any specific cortex or area in this study. Neuronavigation for such studies is advised, and more studies are needed to fully validate the neuroanatomical correlation of other points obtained with the International 10-20 system.

FUNDING

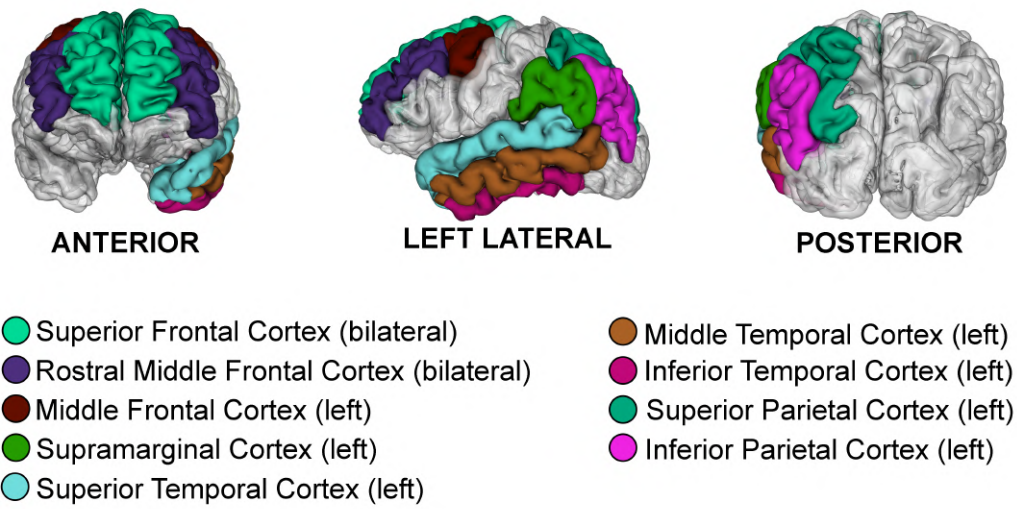
This study was financed, in part, by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Finance Code 001).

DATA AVAILABILITY STATEMENT

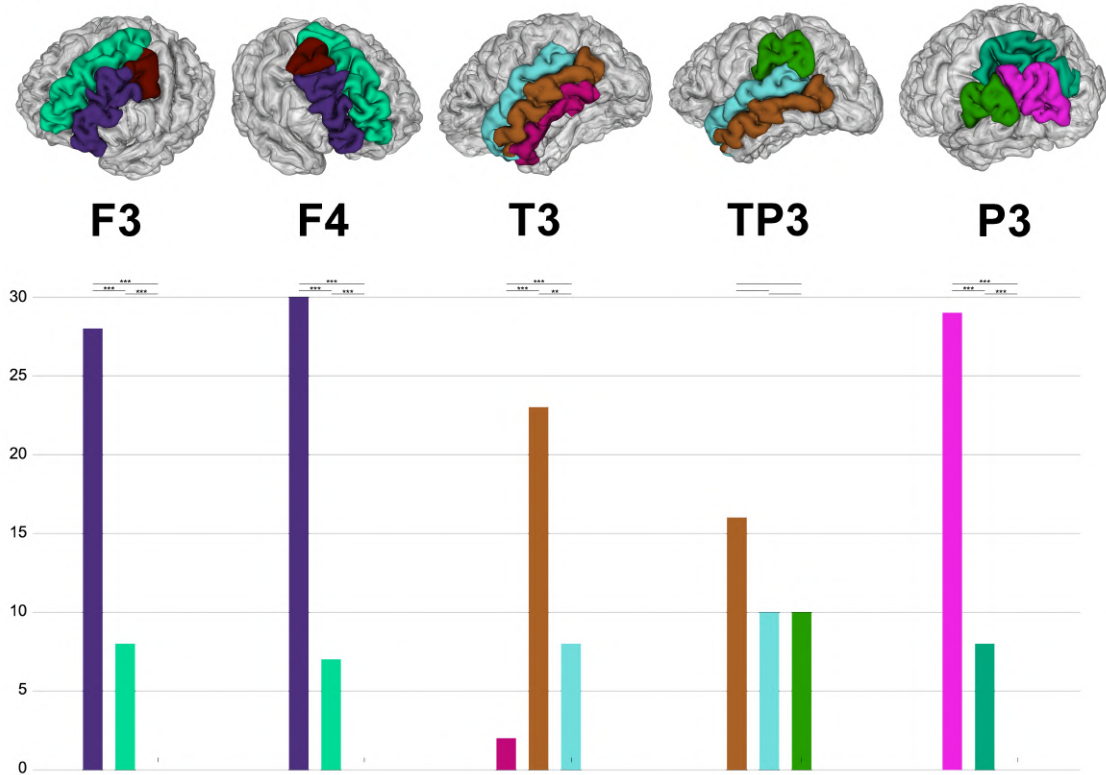
The data of this study may be available under reasonable request.

1 **FIGURE 1**

A

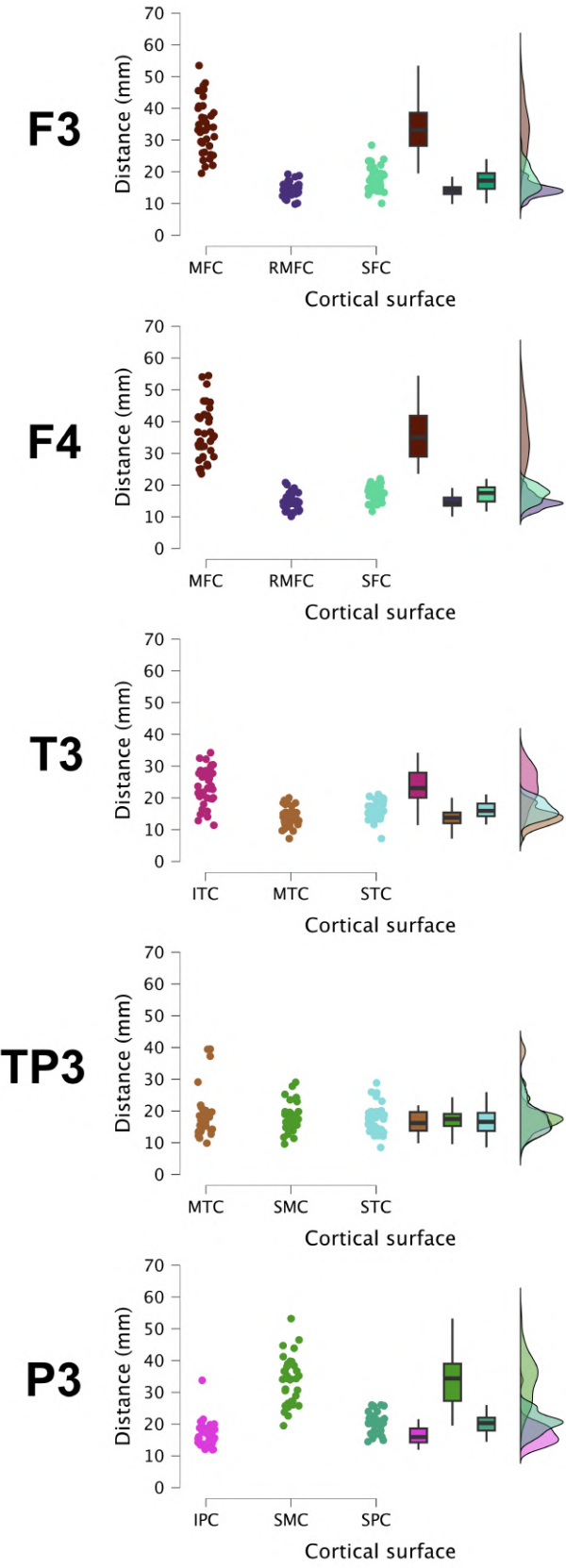


B



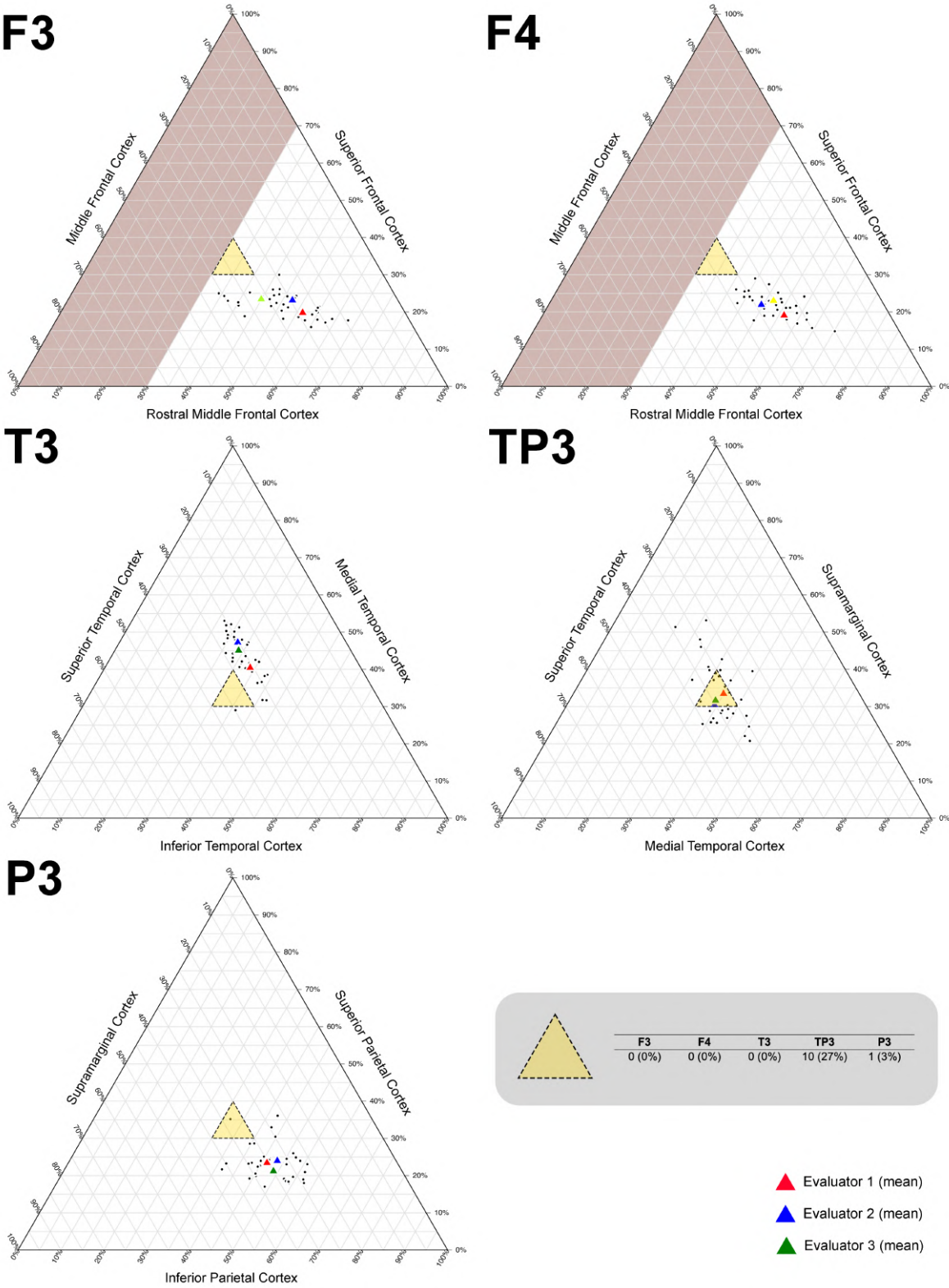
2
3

1 **FIGURE 2**



2

3 **FIGURE 3**



1
2

TABLE 1. Demographics of the participants

Variable	Descriptive	p-value
Sex		
Male	18 (48.6%)	Reference
Female	19 (51.4%)	1.000
Hand dominance		
Right-handed	31 (83.8%)	Reference
Left-handed	3 (8.1%)	<0.001***
Ambidextrous	1 (2.7%)	<0.001***
Unknown	2 (5.4%)	<0.001***
Age	40.27 (9.5)	
Sex	N/A	0.707
Hand dominance	N/A	0.530
Education (Years)	16.62 (3.5)	
Sex	N/A	0.700
Hand dominance	N/A	0.021*

Categorical variables are described as “absolute number (percentage)”; continuous variables are described as “mean (standard deviation)”; * $p < .05$, *** $p < .001$.

TABLE 2. ANOVA Post Hoc Comparisons among absolute distances measured from each 10-20 International System to each correspondent adjacent cortex.

			95% CI for Mean Difference					
			Mean Difference	Lower	Upper	SE	t	p _{tukey}
F3								
	CMF	RMF	19.603	16.624	22.582	1.254	15.636	< .001***
		SFC	16.299	13.320	19.278	1.254	13.001	< .001***
	RMF	SFC	-3.304	-6.284	-0.325	1.254	-2.636	0.026*
F4								
	CMF	RMF	21.571	18.617	24.524	1.243	17.357	< .001***
		SFC	19.069	16.115	22.022	1.243	15.344	< .001***
	RMF	SFC	-2.502	-5.455	0.451	1.243	-2.013	0.114
T3								
	ITC	MTC	9.537	7.247	11.827	0.964	9.896	< .001***
		STC	7.364	5.074	9.655	0.964	7.642	< .001***
	MTC	STC	-2.172	-4.463	0.118	0.964	-2.254	0.067
TP3								
	MTC	SMC	0.348	-2.652	3.349	1.263	0.276	0.959
		STC	1.028	-1.973	4.029	1.263	0.814	0.695
	SMC	STC	0.680	-2.321	3.680	1.263	0.538	0.853
P3								
	IPC	SMC	-17.270	-20.103	-14.436	1.192	-14.485	< .001***
		SPC	-3.661	-6.495	-0.828	1.192	-3.071	0.008**
	SMC	SPC	13.608	10.775	16.442	1.192	11.414	< .001***

CI: Confidence Interval; SE: Standard Error; CMF: Caudal Middle Frontal Cortex; RMF: Rostral Middle Frontal Cortex; SFC: Superior Frontal Cortex; ITC: Inferior Temporal Cortex; MTC: Middle Temporal Cortex; STC: Superior Temporal Cortex; SMC: Supramarginal Cortex; IPC: Inferior Parietal Cortex; SPC: Superior Parietal Cortex; * p < .05, ** p < .01, *** p < .001.

5.4. Protocol study

Once the software mentioned in section 4 was available, the best practices highlighted in section 5.1, the optimized open-source neuronavigation system presented in section 5.2, and the evidence provided in section 5.3 that one can prepare a sonication session with only open-source material and a morphological MRI, it is logical to say that virtually any sonication can be proposed. To follow the objectives proposed in section 3, a non-inferiority, randomized, 1:1, clinical trial is proposed to study the effects of tFUS sonication over the DLPFC versus the therapeutical effect of TMS stimulation over the same cortical area. The justificative and rationale for such a proposal are extensively detailed in sections 1 and 2, and, therefore, will not be redundantly repeated here. It is worth mentioning, however, that the work performed so far allows a wide range of simulations using the tFUS with maximized reproducibility and minimal research funding necessary for neuronavigation. Therefore, although this protocol is a crucial part of this thesis, the results so far are promising to guide future studies with low-intensity tFUS in humans, even if not accompanied by this protocol study.

This study was submitted to the Asian Journal of Psychiatry (ISSN 1876-2018) and accepted for publication in March 3rd, 2024¹⁵⁴.

SAFETY AND EFFICACY OF REPETITIVE STIMULATION OF THE LEFT DORSOLATERAL PREFRONTAL CORTEX USING TRANSCRANIAL FOCUSED ULTRASOUND IN TREATMENT-RESISTANT DEPRESSED PATIENTS: A NON-INFERIORITY RANDOMIZED CONTROLLED TRIAL PROTOCOL

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1 **ABSTRACT**

2 **BACKGROUND:** About 30% of patients diagnosed with major depressive
3 disorder fail with the mainstream pharmacological treatment. Patients that do not
4 achieve clinical remission of symptoms, even with two different antidepressants, are
5 classified with treatment resistant depression (TRD). This condition imposes additional
6 burden with increased Disability Adjusted Life Years. Therefore, complementary
7 treatments are necessary, such as neuromodulation. The transcranial focused
8 ultrasound (tFUS) has emerged in the past few years as a reliable method for non-
9 invasive neuromodulation in humans and may be helpful in the treatment of TRD. The
10 aim of this study is to propose a research protocol for a non-inferiority randomized
11 clinical trial of TRD with tFUS.

12 **METHODS:** Patients with documented TRD will be screened upon their entry
13 into the TRD outpatient clinic at UFMG (Brazil). Fifty-eight patients without clinical
14 history of other psychiatric illness, anatomical abnormalities on magnetic resonance
15 imaging (MRI), or treatment with electroconvulsive therapy will be invited to participate.
16 Patients will be randomized (1:1) in two groups: 1) treatment with a previously
17 established protocol of transcranial magnetic stimulation; and 2) treatment with a
18 similar protocol, using tFUS stimulation. Both groups will attend 7 protocolled spaced
19 days of brain stimulation targeted at the left dorsolateral prefrontal cortex, besides
20 regular consultations in the outpatient clinic. They will also be submitted to 4 sessions
21 of image studies (2 MRI, 2 positron-emission tomography), 3 of neuropsychological
22 assessments (at baseline, 1 week and 2 months after treatment), and the Hamilton
23 Scale for Depression to analyze the severity of depressive symptoms.

24 **DISCUSSION:** This clinical trial intends to verify the safety and clinical efficacy
25 of tFUS stimulation of the dorsolateral prefrontal cortex of patients with TRD, in
26 comparison with a previously established method of neuromodulation.

27 **Keywords:** transcranial focused ultrasound, treatment-resistant depression,
28 dorsolateral prefrontal cortex, non-invasive brain stimulation

WHAT IS ALREADY KNOWN ON THIS TOPIC

The transcranial focused ultrasound (tFUS) has been studied over the past decade with positive results for neuromodulation in humans. The main characteristics that make it a promising therapeutic device in the future include its capacity of reaching deep brain structures, its high spatial specificity and its non-invasive profile, when used in low intensities. So far, however, most studies using the tFUS for neuromodulation focused on healthy subjects and/or single applications, raising the need for future clinical trials including patients with specific medical conditions.

WHAT THIS STUDY ADDS

The main goal of this clinical trial is to evaluate the safety of multiple, protocolled, and controlled applications of tFUS aiming neuronavigation. Besides multiple clinical assessments, neuroimaging (both functional and morphological) will also evaluate possible changes in the brain after sonication. The other purpose of the study is to evaluate the clinical efficacy of tFUS stimulation over the dorsolateral prefrontal cortex, which is already targeted in other non-invasive methods of neuromodulation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

This study has the potential to reveal a safe protocol of tFUS stimulation in humans, opening new approaches for interventions in patients with neuropsychiatric disorders. Besides, it can also reveal the therapeutic efficacy of a novel technology in patients with treatment-resistant depression, which is considerably challenging in the clinical practice.

1 INTRODUCTION

2 Background and rationale

3 Major depressive disorder (MDD) is considered a global health problem,
 4 affecting approximately 10% to 15% of the population annually⁶⁵. About 30% of
 5 patients fail to achieve full symptomatic recovery even with multiple pharmacological
 6 attempts⁷⁰, and can be described as having treatment-resistant depression (TRD). A
 7 recent consensus defined TRD by a considerable lack of response (less than 25%) to
 8 at least 2 antidepressants⁷².

9 Currently, new therapeutic approaches have been used for the treatment of
 10 TRD, with emphasis on the use of augmenting pharmacotherapies, electroconvulsive
 11 therapy (ECT), transcranial magnetic stimulation (TMS) and intranasal esketamine¹⁵⁵.
 12 However, therapeutic modalities such as ECT and esketamine may be considered
 13 invasive, given the need for anesthesia, vital signs monitoring during the treatment
 14 session and collateral effects.

15 In the context of TRD, a therapeutic approach that raises an increasing interest
 16 is neuromodulation, which is defined by facilitation or inhibition of neuron activity in a
 17 specific brain area¹⁶. The most well-established method of non-invasive
 18 neuromodulation is TMS, which has been studied in multiple settings of MDD, including
 19 TRD⁵³. TMS's major advantages is its feasibility in the outpatient setting without need
 20 of anesthetic agents. However, its application is limited to cortical areas, and it is
 21 unable to reach deep-brain structures. One of the approaches for the use of TMS is
 22 the intermittent theta-burst stimulation (iTBS). The benefits of iTBS include shorter
 23 sessions' length and a similar efficacy when compared with classic TMS protocols¹⁵⁶.

24 The left dorsolateral prefrontal cortex (L-DLPFC) is considered directly related
 25 to depression pathogenesis, and is a core focus of TMS stimulation. Previous data
 26 supports the hypothesis that stimulating the L-DLPFC may be beneficial in the
 27 treatment of MDD and TRD⁶⁰.

28 In the context of non-invasive neuromodulation, the transcranial focused
 29 ultrasound (tFUS) has received increasing attention during the past decade³⁸. It
 30 consists in delivering ultrasonic waves through the skull using low acoustic intensity,
 31 which has been proven effective in modulating neuron activity in animals and humans.
 32 Although the exact mechanism of neuromodulation using tFUS is uncertain, it has been
 33 hypothesized that it is related to mechanical tissue displacement, or cavitation between

the layers of the neuron cell membrane¹¹. The effects of thermal rise in neuromodulation are still unknown, since low-intensity ultrasounds are thought to elicit little temperature variation, in contrast with high-intensity ones. However, the attenuation of the ultrasonic wave invariably leads to thermal energy deposition, that might not be neglected, since even slow thermal variations may cause modulation of neural activity¹³. Regardless of the mechanism of action of the modulatory effect of low-intensity focused ultrasound in the nervous system, it is considered to be transient and non-ablative¹⁴, and generally considered safe.

The intensity of tFUS aiming neuromodulation is limited by the United States Food and Drug Administration (FDA) by 3 major safety parameters. They are the mechanical index (MI), spatial-peak temporal average (I_{SPTA}), and spatial-peak pulse average (I_{SPPA}). The MI is defined as the derated peak rarefactional pressure (measured in MPa) divided by the square root of the acoustic frequency⁵. I_{SPTA} is defined as the pulse intensity times the pulse repetition frequency, and I_{SPPA} is defined as the pulse intensity divided by the pulse period⁸⁹. The FDA considers brain sonication to be safe if $I_{SPTA} \leq 720 \text{ mW/cm}^2$, $I_{SPPA} \leq 190 \text{ W/cm}^2$ and $MI \leq 1.9$. Most studies published so far respected the FDA power limitations at the sonication focus³⁸.

In contrast with TMS, tFUS has both a high spatial specificity and the capacity to reach deep brain structures, which may be fundamental in neuromodulation of small areas. Although a single sonication with tFUS in humans seems to be safe³⁸, to the best of our knowledge, few is known about its safety profile in repetitive sessions, which seem to be fundamental in neuromodulating depressed patients. Moreover, to the best of our knowledge, no previous study measured the safety and clinical efficacy of tFUS in TRD.

Choice of comparators

Most studies using tFUS in humans so far have done so with either the own patient as his comparator or with a sham group which was not sonicated, or sonicated in a different area. As this study aims to analyze both safety and clinical efficacy of tFUS, both approaches will be used. Safety will be measured by a standardized questionnaire (furtherly discussed) and clinical efficacy will be compared to the one of TMS, in a protocol used in previous clinical trials. As there is few evidence about the protocol of sonication using tFUS, its protocol will mimic the TMS arm.

Objectives

The objectives of this clinical trial are: (1) to investigate the safety of use of the tFUS in patients with TRD in a repetitive protocol; (2) to analyze the efficacy of intermittent tFUS sonication to the L-DLPFC, in comparison with a previously established protocol of intermittent TMS sonication; and (3) to analyze differences in the response of intermittent tFUS sonication according to skull thickness, sub-segmented brain area under sonication, medications used, socioeconomical status, and clinical characteristics.

Trial design

This trial is designed as a randomized, controlled, experimental, single-center, non-inferiority trial, with two-arm parallel groups. Randomization will be performed as simple randomization with a 1:1 allocation.

Ethics approval

This study was approved by the institutions Ethics Committee (number CAAE 56451022.1.0000.5149, Universal Trial Number U1111-1293-4994) and will be submitted to Brazilian Registry of Clinical Trials.

METHODS: Participants, interventions, and outcomes

Eligibility criteria

The participants inclusion criterion is: patients aged 18 to 60 years old with diagnosed TRD (either via clinical records or via validated psychometric questionnaires), defined as treatment failure (< 25% in symptom improvement, measured using the Hamilton Scale for Depression) in patients with a baseline score of at least 20, after at least 2 antidepressant trials with adequate dosage (as approved by the FDA) and timing (6 weeks). The participants exclusion criteria are: other severe mental disorders, such as bipolar depression and/or schizophrenia; brain anatomical abnormalities identified in the magnetic resonance imaging (MRI); previous treatment trial with ECT or TMS; and clinical characteristics that restrain tFUS sonication, such as scalp scar or voluminous hair. All patients must sign the consent form to be enrolled in the clinical trial.

Interventions: TMS group

The treatment in the TMS group will follow a previously described protocol of iTBS used in a recent clinical trial¹⁵⁶. It will consist of 7 treatment days, each with 3 sessions per day set apart by 15 minutes (21 sessions total). The treatment days will be divided into 4 weeks. iTBS will involve the application of 3 TMS pulses at 50 Hz every 200 msec (5 Hz). The delivery of TMS pulses will last for 2 s, followed by 8 s interval (10 s total each cycle), for a total of 190 s. Stimulation intensity will also follow previously described data, at 120% of the resting motor threshold. The TMS pulses will be applied to the scalp adjacent to the L-DLPFC, which will be found using the Beam F3 method⁶¹ for the first session.

Interventions: tFUS group (general protocol)

The tFUS applications will follow the same week schedule and stimulation timing of the TMS arm (delivery of 3 sessions per day, spaced by 15 minutes, lasting for 190 s each). This timing of 190 s per burst is considered safe, as recent studies have used as long as 20 minutes of continuous tFUS stimulation without reporting major adverse effects⁷⁵. Due to intrinsic differences between the mechanism of action of TMS and tFUS, though, the time setting within each burst will be different: this study will follow the only repetitive protocol published so far, to the best of our knowledge⁸⁰, which consists of pulse repetition frequency = 500 Hz, pulse duration = 0.5 s, and duty cycle = 50%. The inter-stimulus interval will follow the study that described the longest tFUS stimulation period⁷⁵ and will be set at 5 s, resulting in 38 tFUS stimulations per burst session (total 798 stimulations in the whole treatment). The stimulation scheme for both arms is illustrated in Figure 1.

This clinical trial will not assume skull attenuation of acoustic power. This is due to the high number of repetitive stimulations at the same target, and to the heterogeneity of skull thickness, porosity, and consequent attenuation among patients and different skull regions. In contrast with previous research in humans, which did not aim specifically the L-DLPFC, a recent study calculated different attenuations and found an attenuation of only 18.2% by the skull overlaying the L-DLPFC¹⁰⁶. Therefore, this study will use a cautious protocol, considering the possible accumulative effect of multiple stimulation sessions. All the calculations will respect the FDA limits.

Interventions: neuronavigation

Neuronavigation is a real-time procedure that allows the visualization of brain targets during the neuromodulation session. In this study, a modified version of a previously open-source neuronavigation tool⁸⁶ will be used. The core software employed in this study are: (1) 3D Slicer version 5.4.2¹¹⁸, (2) FreeSurfer suite version 7.3.2¹¹⁹, and (3) Plus toolkit version 2.8.0¹²⁰. The hardware will be composed of (1) a Windows 10 computer with 16GB-RAM and a 2.80 GHz processor, (2) an NDI© Polaris Vicra stereotactic infrared camera (Ontario, Canada), and (3) a set of 3D-printed devices with infrared-reflective spheres. A novel two point-based neuronavigation was specifically designed to this study due to its repetitive protocol. The whole process of neuronavigation is described in Supplementary File 1, along with Figures 2, 3 and 4.

Outcomes

Primary outcomes: (1) qualitative improvement in symptom severity, defined as a reduction of 25% and less than 50% (partial response) or 50% (response) in symptoms' severity or remission (defined as scoring ≤ 7 in the HAM-D or ≤ 10 in the MADRS), as measured by the MADRS and QIDS-SR₁₆ at the end of the treatment and 2 months after treatment; and (2) clinical safety, as weekly measured by the translated version of the Udvalg for Kliniske Undersøgelser Side Effect Rating (UKU) Scale, which has already been used in previous clinical trials with Brazilian patients¹⁵⁷.

Secondary outcomes: (1) quantitative change in baseline depression symptoms' severity, as measured by the Hamilton Scale for Depression (HAM-D)¹⁵⁸, the Montgomery-Åsberg Depression Rating Scale (MADRS)¹⁵⁸ and the self-reported Quick Inventory of Depressive Symptomatology (QIDS-SR₁₆)¹⁵⁹, which will be applied at baseline, weekly during treatment, and 2 months after treatment; (2) quantitative changes in neuropsychological assessments, performed at baseline, after treatment and 2 months after treatment; (3) quantitative changes in ¹⁸F-fluorodeoxyglucose uptake in the PET scan, performed at baseline, and immediately after treatment, protocolled as previously described¹⁶⁰; (4) quantitative assessment of treatment efficacy according to clinical and socioeconomical data; (5) quantitative assessment of treatment efficacy according to data acquired in image studies, such as different sonicated areas within the L-DLPFC and skull bone thickness at the site adjacent to

the neuromodulation device (either TMS or tFUS); and (6) dropout rate in each group for any reason.

Sample size

The sample size was calculated using the G*Power software (Düsseldorf, Germany, version 3.1)¹⁶¹, considering a one-tailed analysis with $\alpha = 0.05$, power = 0.8 and non-inferiority limit = 16%. The effect size was determined by previous data of efficacy of repetitive TMS for major depression (33.6%)⁵⁴. The total sample size was calculated as 194 (2 x 97 per group).

METHODS: Assignment of interventions

Allocation

Participants will be randomly assigned to either control or one of the experimental groups with a 1:1 allocation as per a computer-generated randomization schedule. The allocation sequence will be generated applying a permuted block design with random blocks.

Implementation

All patients who give consent for participation and fit inclusion/exclusion criteria will be randomized. Randomization will be requested by a staff member and will be performed by an independent member of the research group. This member will then upload the results to the institution's database in Research Electronic Data Capture (REDCap)¹⁶². More information about the database will be detailed later in the protocol. The neuromodulation researcher will have access to the patient's allocation only in the pre-specified day for the first neuromodulation session. Staff responsible for recruitment and symptom ratings is not allowed to receive information about the group allocation. The schedule of interventions, scales and appointments is illustrated in Figure 5.

Blinding

It is a single-blinded trial (outcomes assessor) and, during the treatment, the patient will be seen by two different teams: (1) the assistant physicians of the outpatient clinic and (2) the neuromodulation team, who will perform either the TMS or the tFUS

intervention. There will be no members working simultaneously on both teams. The results of randomization will be available only to the neuromodulation member responsible to the intervention, and the scales' results will be available only to the outpatient clinic workers. Medication will be kept stable during the treatment period, and dropout will be considered for any reason. The neuropsychological assessments' results will be held in a different project in REDCap, and will be available only to the neuropsychology team for final statistical analysis.

METHODS: Data collection, management, analysis

Data collection

An initial screening will be carried out by a psychiatrist of the TRD outpatient clinic. In this moment, the patient will be assessed by the mini-international neuropsychiatric interview (MINI Plus)¹⁶³ to rule out psychiatric diagnosis included in the exclusion criteria. In addition, other scales will be assessed, including the World Health Organization Quality of Life abbreviated measure (WHOQOL-Bref)¹⁶⁴ and socioeconomic data.

Following inclusion in the study and signature of the consent form, the patients will be allocated to either tFUS or TMS stimulation. Blocks of 8 patients will be randomized to each group. Immediately after randomization, the patients will be invited to pre-interview assessments, including the neuropsychological evaluation and imaging studies, as mentioned in Figure 1. After MRI availability and pre-processing, the patient will be invited to initiate the intervention, and will know which arm they were assigned to. Safety will be evaluated in each intervention day using the UKU scale by the intervention applicator, while depression severity scales will be evaluated by the main caregiver or a trained member of the outpatient clinic. If any major side effect is observed during treatment by the physician administering the intervention, it will be interrupted, and the patient will be withdrawn from the study.

After finishing the treatment protocol, the patients will be invited to another block of neuroimaging and neuropsychological assessment. They will also be followed up in the refractory depression ambulatory for at least 2 months. After this period, they will be invited to another neuropsychological assessment, and depression severity scales will be collected again.

1 Data management

2 As mentioned before, all data in this study will be stored using REDCap, which
 3 is a specific software that allows blinding of different groups and increase the patient's
 4 data safety. At least four different projects will be created to avoid storage of data in
 5 only one database, and provide each team with only the necessary information: (1) an
 6 identification project, with sensible information, available only to the study staff, used
 7 to encode the patients in the other projects; (2) a neuropsychological project,
 8 composed of the main scales of the neuropsychological assessment, and its final
 9 report; (3) a clinical project, available to the outpatient clinic team, aimed at collection
 10 of data from the depression severity scales; and (4) an intervention project, which will
 11 be used to register every treatment day and the UKU scale. Participant files will be
 12 maintained in storage for a period of 10 years after completion of the study.

14 Statistical methods

15 Data analysis will be carried out using the Statistical Package for Social
 16 Sciences software – SPSS version 21, formalizing information that can be presented
 17 in the form of graphs, tables, or charts to favor the process of understanding and
 18 discussion. The main analysis will be performed by quantitative evaluation of both UKU
 19 scale and depression severity scales, as well as qualitative evaluation of remission
 20 rates right after treatment and maintenance 2 months after its end, using the Fisher's
 21 Exact test or the Two-Proportion Z-Test. Confidence interval of 95% and p value <0.5
 22 will be established. Participants who do not complete the intervention or misses the
 23 post-intervention evaluation will be excluded from the study or will have their data
 24 separately analyzed in sub-groups.

26 Confidentiality

27 All study-related information will be stored securely at the study site. All
 28 participant information will be stored in locked file cabinets in areas with limited access.
 29 All data collection forms will be identified by a coded identification number only to
 30 maintain participant confidentiality. All local databases will be secured with password-
 31 protected access systems.

33 DISCUSSION

About one third of patients with MDD fails to respond to optimized pharmacological treatment and may be characterized as having TRD⁷⁰. Differently from MDD, TRD still lacks standardization of best practices and often requires neuromodulatory techniques, including ECT and TMS.

In this scenario, tFUS has been studied as a promising technique for neuromodulation, which combines both the ability to reach deep brain structures and precise spatial resolution³⁸. Although most studies published so far focused on the neuromodulatory capacities of tFUS, most of them consisted of single sonications and, so far, few has been studied about its effects on the L-DLPFC. Previous studies have shown tFUS's ability to both stimulate and inhibit regions of the human brain, which may be beneficial in clinical practice. However, the currently available neuromodulation methods for TRD usually consist of repeated sessions, which, to the best of our knowledge, has not been tested with tFUS on humans yet.

Besides the safety concerns that arise with the repetitive protocol, it is also challenging due to neuronavigation issues. Previously described techniques of open source neuronavigation help to establish the real-time focus of the transducer inside the brain, which is undoubtedly helpful (and, maybe, indispensable) in single sonications. However, due to the cigar-shaped area of tFUS stimulation, a rigorous method of neuronavigation is needed for repeated sessions.

The L-DLPFC is the main target of TMS due to its apparent direct relationship with depressive symptoms⁶⁰. Although TMS does not necessarily need neuronavigation to be applied clinically, it is recommended by some authors to reduce mismatches in the placement of the coil and guarantee reproducibility, especially in the research environment.

In the case of tFUS, neuronavigation is mandatory for deep brain stimulation. Although this protocol targets a superficial brain area, it also demands rigorous steps to keep the same area sonicated through time. Therefore, the 2-point method will be used to reduce possible mismatches of tFUS stimulation in this protocol.

FUNDING

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1 **COMPETING INTERESTS**

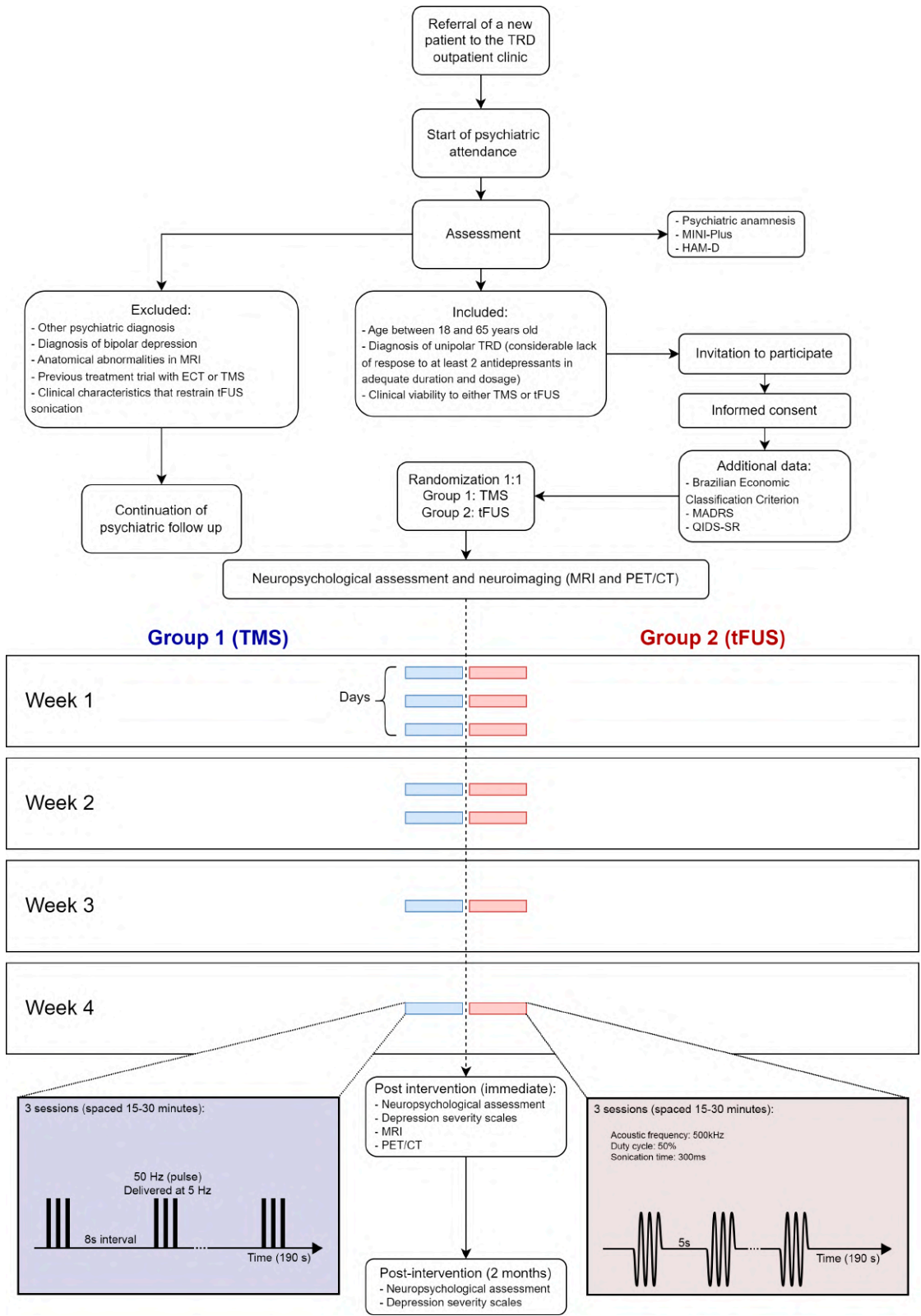
2 The authors declare no conflicts of interest.

3

4 **ACKNOWLEDGEMENT**

5 None.

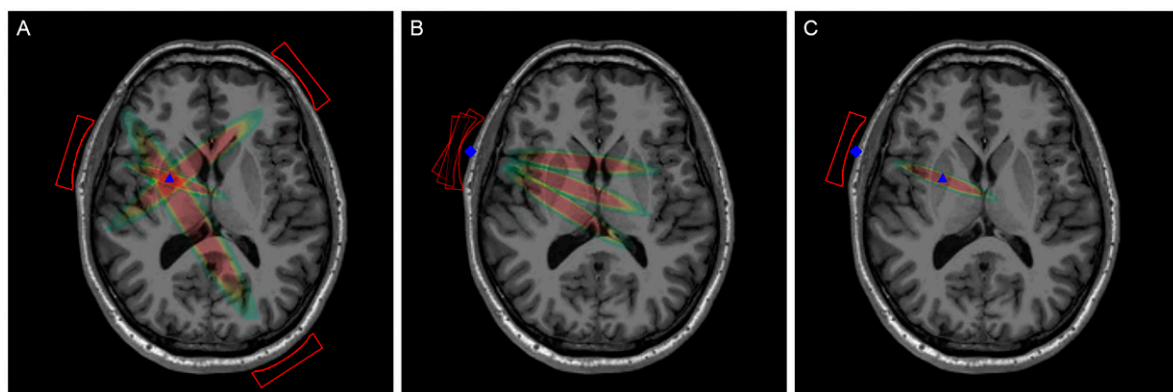
1 **FIGURE 1.**



2

3

4 **FIGURE 2.**

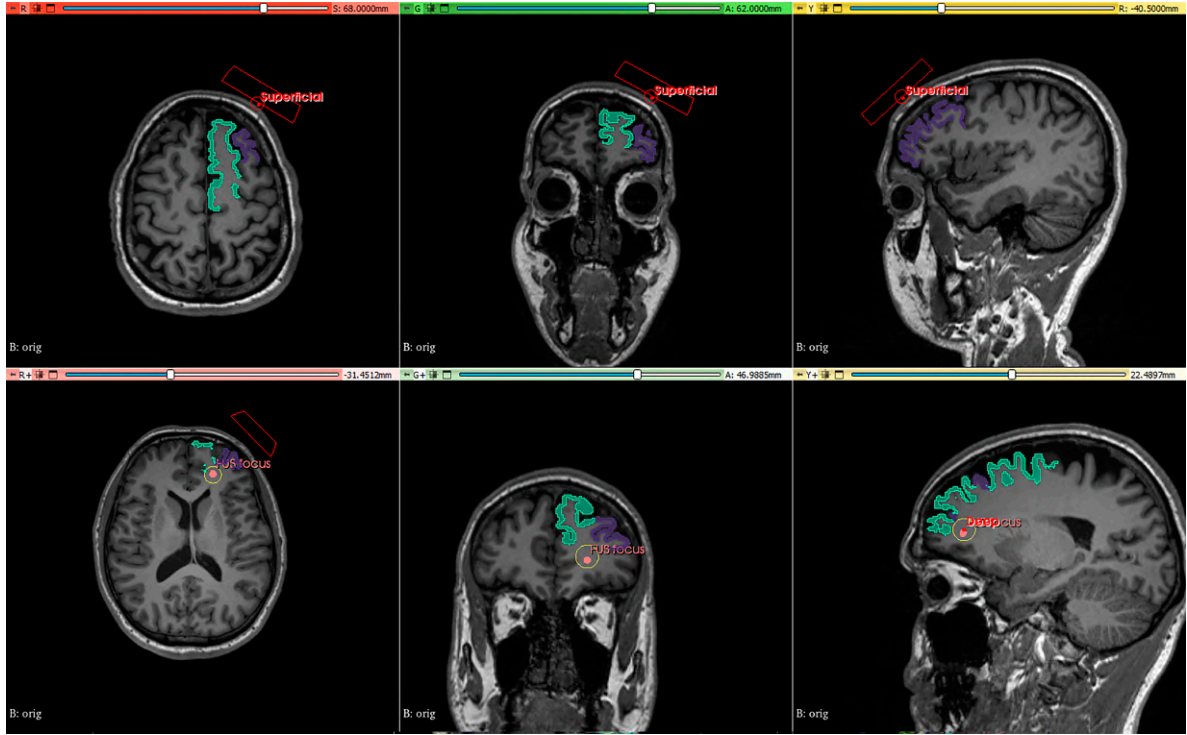


1

2

1 **FIGURE 3.**2
3

1 **FIGURE 4.**



2

3

1 **FIGURE 5.**

	Study period								
	Enrollment	Allocation	Pre-intervention	Neuromodulation (t3)				Post-intervention (immediate)	Post-intervention (2 months after)
TIMEPOINT	-t1	0	t1	Week 1	Week 2	Week 3	Week 4	t4	t5
Referral (to the outpatient clinic)	X								
Eligibility screen	X								
Informed consent/assent	X								
Allocation		X							
Neuropsychological assessment			X					X	X
PET/CT			X					X	
MRI			X					X	
Interventions* (either tFUS or TMS stimulation)				3 days	2 days	1 day	1 day		
HAM-D	X			X	X	X	X	X	X
MADRS	X			X	X	X	X	X	X
QIDS-SR	X			X	X	X	X	X	X

2

3

5.5. Case report

Ideally, a clinical trial proposal must consider all available data, including positioning the patient, methods to avoid loss of acoustic energy, and protocolized metrics. In practice, these details are either (1) omitted from the protocol studies, (2) based on robust previous data that allows maximum precision in the method description, or (3) tried on pilot studies that allow fine corrections without compromising the general objective of the study. As section 5.1 shows, limited data about those details in previous studies is available. Therefore, multiple tests were performed with volunteer researchers to optimize how the protocol would be best transcribed into reality, allowing a detailed description without robust previous literature data. Extensive documentation of this methodology refinement is provided in Supplementary File 1.

To verify that the method could be performed by multiple operators, in a clinical setting that would be replicated in the clinical trial, a pilot case is reported. It suggests that the methods described in sections 5.2, 5.3, and 5.4 are feasible in clinical practice and that repeated sonication over the DLPFC using tFUS in humans may be accurately performed with an optimized learning curve.

**SAFETY AND TOLERABILITY OF REPETITIVE LOW-INTENSITY TRANSCRANIAL
FOCUSED ULTRASOUND STIMULATION OVER THE DORSOLATERAL
PREFRONTAL CORTEX IN A PATIENT WITH TREATMENT-REFRACTORY
DEPRESSION: A FIRST-CASE REPORT**

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1. CASE REPORT

Dear Editor,

Your journal recently published a full research article about the effects of low-intensity transcranial focused ultrasound (tFUS) stimulation over the frontal cortex on symptoms related to depressive disorders¹⁶⁵. We are conducting a Clinical Trial (Universal Trial Number U1111-1293-4994) using a similar approach. A pre-established, 4-week-long, repetitive protocol is being used to analyze the safety, tolerability, efficacy, and clinical reliability of tFUS stimulation for patients with treatment-resistant depression (TRD). So far, there is a gap in the literature about repetitive tFUS stimulations³⁸, hence the need for studies that follow a specific protocol. However, unlike the approach used in the abovementioned paper published in your journal, we repeatedly use a facilitation protocol to stimulate the left dorsolateral prefrontal cortex (DLPFC).

We report the case of Mrs. A, a 38-year-old woman, who was enrolled in the abovementioned Clinical Trial on July 26th 2023, and was the first patient assigned to the tFUS arm. At baseline, she was staged as level 2 in Thase and Rush¹⁶⁶ and Massachusetts General Hospital¹⁶⁷ stratifications, with a HAMD-17¹⁶⁸ score of 32, a MADRS¹⁶⁹ score of 21), and QIDS-SR score of 24¹⁷⁰. The Mini-International Neuropsychiatric Interview¹⁶³ was applied and tested positive for current and past depressive episodes, high current suicide risk, agoraphobia, social phobia, current panic disorder, and tobacco use dependence. She was referred to the Refractory Depression Outpatient Clinic due to refractoriness to combined therapeutic doses of Venlafaxine, Bupropion, Nortriptyline, and Lithium. Antidepressants were titrated out and Tranylcypromine was initiated on September 18th 2023 at 20mg/day. It was further adjusted to 40mg/day on December 20th 2023, and were kept stable during the trial. Alprazolam was kept at a dosage of 2mg/day since the patient was enrolled.

Functional magnetic resonance (MRI), positron-emission tomography associated with computerized tomography (PET/CT) images, and neuropsychological evaluations were performed before and after the procedure. The patient underwent twenty-one sessions of low-intensity tFUS stimulation over the left DLPFC using a facilitation protocol. The stimulation began on December 19th, 2023, and finished on January 10th, 2024. Scores from HAMD-17, MADRS, and QIDS-SR are depicted in Figure 1A.

The sonication was performed using a transducer from NeuroFUS PRO®, as described in the clinical protocol elsewhere¹⁵⁴. Minor details, such as comfortable positioning for both the patient and applicator, were addressed during the study. In our experience, laying the patient in a reclinable chair exposes the temporal region and makes it easier to identify the F3 point. An articulated extension rod was essential to keep the transducer fixed since the hydrogel needed for sonication leads to easy undesired movement of the transducer. The best position for the applicator was in orthostatism, keeping the left hand over the transducer to avoid loss of contact with the scalp and the right hand gently holding the patient's head. The use or not of music during the stimulation and intervals were up to the patient's decision. In this case report, the patient opted for silence and minor interactions.

Safety was evaluated weekly and one week after the intervention, using the Udvalg for Kliniske Undersøgelser Side Effect Rating (UKU) Scale¹⁵⁷, which evidenced self-related daytime sleepiness/sedation (item 1.3) of degree 2/3 in weeks 1 and 2, and 1/3 in weeks 3 and 4. It also evidenced headache (item 2.9) in of degree 2/3 in week 2, with no other alterations.

It is worth mentioning that the patient divorced during the trial, which was a considerable stressful event, but did not motivate either prescription changes or withdrawal from the trial. For this reason, the specific moment the event happened is pointed out in Figure 1A. Psychiatric, psychological, and clinical referrals were available throughout the whole trial period.

2. DISCUSSION

To the best of our knowledge, this is the first case report of a patient with TRD who underwent repetitive low-intensity tFUS stimulations over the left DLPFC in a protocolled setting. Although no clinical improvement (defined by at least a reduction of 25% in validated scales), was formally observed⁷², some improvements could be perceived: (1) a global low glucose uptake was seen at baseline but was improved (defined by a variation <1 standard deviation in the z-score) in all areas. Moreover, the left mesial temporal cortex, whose connection to the DLPFC was already described¹⁷¹, was considered hyperactive post-intervention; and (2) although modest, the neuropsychological evaluation showed some improvement in aspects of language, attention, and processing speed, which are associated with the left mesial temporal

1 cortex¹⁷². This suggests a possible anatomic-psychological correlation that, so far, has
2 not been described by tFUS stimulation reports.

3 The global improvement in glucose uptake (Figure 1C) was an unexpected
4 result of this intervention. Not surprisingly, the left and right prefrontal cortices were the
5 fourth and third areas that showed the most significant improvement, respectively. Both
6 sides showed more improvement in sensorimotor and occipital areas than in the
7 prefrontal area, which may be attributed to intrinsic changes in ocular motion during
8 the PET/CT scan. However, a consistent positive delta in the z-score on both sides,
9 associated with the global normalization of low glucose uptake, cannot be
10 undervalued.

11 Tolerability was a central concern due to the intervention's first-case setting. It
12 is noteworthy that this patient was under Tranylcypromine treatment, which causes
13 severe side effects in about 21% of patients¹⁷³. The Tranylcypromine optimization from
14 20mg to 40mg occurred at the beginning of the intervention, theoretically raising a
15 concern of side effect confusion, which was not seen on the UKU scale's results.
16 Moreover, she experienced a significant stressful event during the intervention, which
17 did not motivate her to drop out.

18 A recent study reported "sleepiness" as a consistent side effect of tFUS
19 sonication¹⁷⁴, while a recent review only mentioned "headache" as a mild side effect
20 among individual studies¹⁷. Therefore, both side effects were consensually considered
21 as "possible" in the UKU scale. The use of this scale was justified due to the lack of a
22 specific scale for adverse effects after tFUS sonications, even though previous studies
23 have converged into common adverse events.

24 Therefore, although possible side effects may occur in repetitive tFUS
25 stimulation over the left DLPFC, this case report suggests the stimulation is tolerable
26 and safe even when combined to a Monoamine Oxidase Inhibitor. More studies are
27 necessary to assess the safety and tolerability of tFUS sonication in clinical practice.

28 The best contribution of this case report is probably demonstrating that a
29 repetitive protocol can be achieved using low-intensity tFUS with an optimized open-
30 source neuronavigation method⁸⁶. Although modest, the pilot study suggests that the
31 methodology used can be reapplied in other studies and, in the future, in clinical
32 practice. The description of the method used is frequently a gap in the literature, and
33 this case report tried to describe how the repetitive sessions were performed to its best.

2.1. Limitations

Although the prescription was kept stable during the trial, the dose adjustment of Tranylcypromine may have played a role in the abovementioned findings since its full therapeutic effects may take weeks. If the image and neuropsychological findings were due to this schedule coincidence, they would probably be absent in the final analysis of the clinical trial. The lack of anatomic-psychological correlation with clinical improvement, however, is up for debate and needs further studies to be addressed, even though the depression's bio-psycho-social model may explain part of it.

Factors such as low socioeconomic status, tobacco dependence, low education level, and family conflicts (which were all present in this patient) may have played a role in the stressful event and were already mentioned as risk factors for depressive symptoms¹⁷⁵.

Another limitation of this short communication is the narrative of a single patient who suffered a significant stressful event during the clinical trial and had multiple comorbidities triaged by the MINI-Plus. The use of benzodiazepines may have impaired the safety evaluation, and since this was the first patient of a large clinical trial, minor adjustments needed to be made during the intervention. These details, albeit maybe considered "minor", need to be stated and carefully evaluated.

3. CONCLUSION

This is the first report of a repetitive protocol of low-intensity tFUS stimulation over the left DLPFC in a patient with TRD. It provides insights into the possible effects of such methods and stimulates further research in the area, particularly using multiple sonications over the same area to achieve long-term effects. Additional studies with repetitive sessions of low-intensity tFUS stimulations are encouraged since this pilot suggests they are safe and may be efficacious. The intervention was considered well tolerated, especially due to concomitant Tranylcypromine treatment. Furthermore, the growth of tFUS research and the lack of a specific scale for addressing adverse effects are considerable rationales for the development of such a scale.

FUNDING

1 This study was financed, in part, by the Coordenação de Aperfeiçoamento de
2 Pessoal de Nível Superior. The National Institutes of Science and Technology in
3 Responsible Neurotechnology received funding from the Conselho Nacional de
4 Desenvolvimento Científico e Tecnológico (Brazil, funding number: 406935/2022-0).
5 The tFUS device used in this trial was acquired with resources from the Fundação de
6 Amparo à Pesquisa do Estado de Minas Gerais (Brazil, process number: APQ-00735-
7 19).

9 **DATA AVAILABILITY STATEMENT**

10 The data of this study may be available under reasonable request.

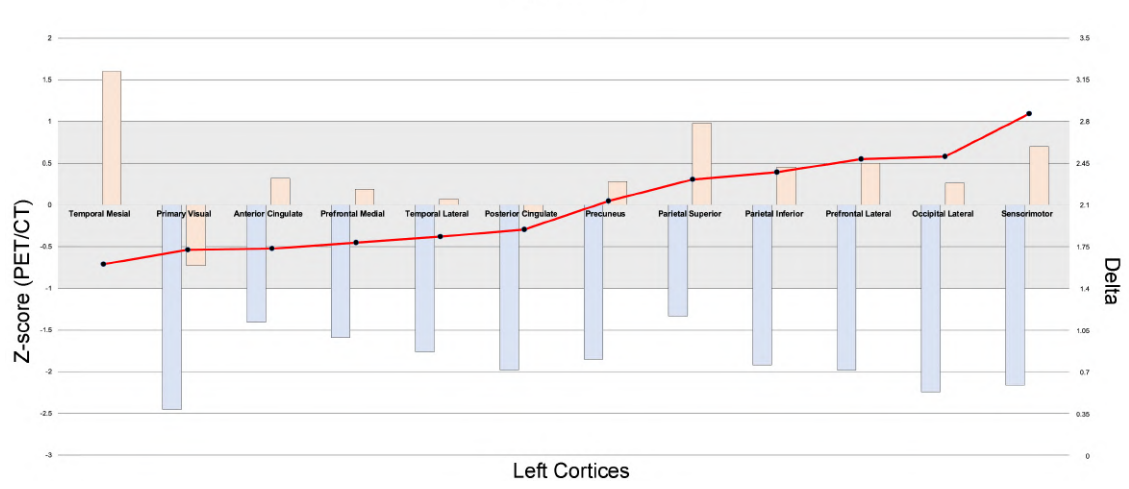
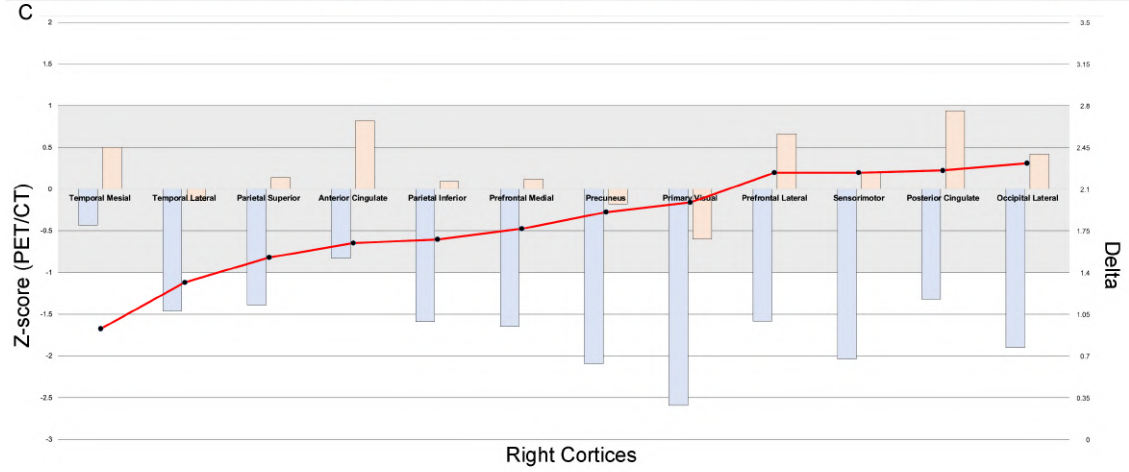
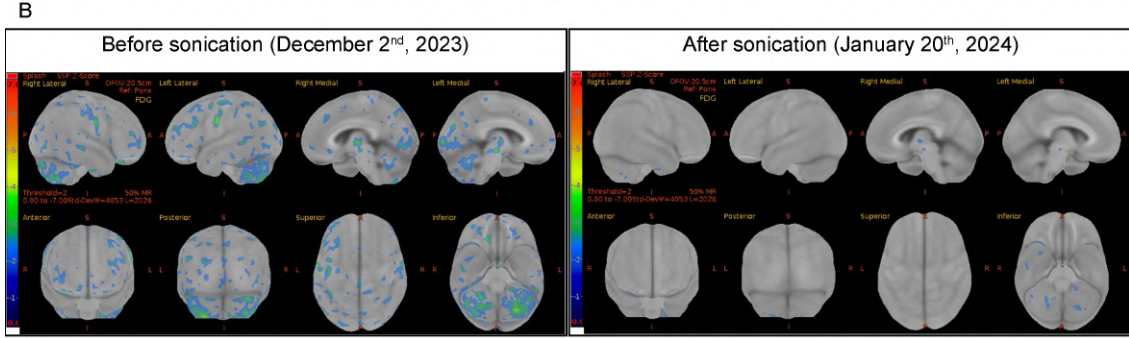
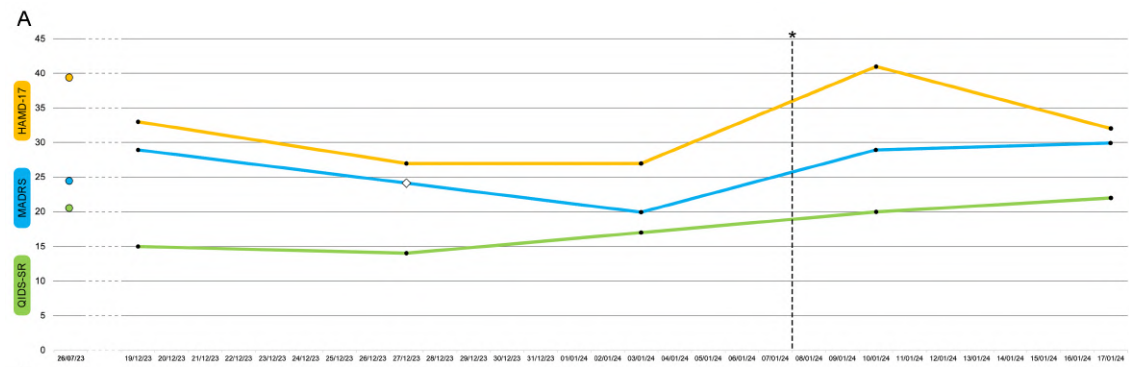
12 **ACKNOWLEDGEMENTS**

13 None.

15 **CONFLICTS OF INTEREST STATEMENT**

16 The authors declare no conflict of interest.

1 **FIGURE 1**



6. DISCUSSION

The studies aiming for neuromodulation in humans with low-intensity tFUS started about a decade ago⁷³ and have become more complex and sophisticated. Yet, this is still a developing field that lacks standardization, more robust studies, research with patients with neuropsychiatric conditions, and refined methodologies. In this study, these gaps in the literature were addressed to propose a robust clinical trial with comparators, scales, and settings that were carefully selected by theoretical guidance.

For such guidance, it was necessary to perform a systematic review (section 5.1). It found no previous studies with low-intensity tFUS for depression, although previous studies have already pointed out that it could be an alternative in the future¹⁷⁶. Surprisingly, one study used an inhibitory protocol to the right side of the prefrontal cortex⁸¹, with positive mood results, which somewhat mimics TMS protocols⁵⁰. The systematic review was fundamental for raising important details on acoustic frequency, sonication parameters, and loci of stimulation used in previous studies. It was the basis for establishing a stimulation protocol with detailed parameters to facilitate (instead of inhibiting) neurons in a specific area. Moreover, the study provided information that most of the previous research with low-intensity tFUS considered it “safe”, although few used protocolled scales, and none mentioned it in a repetitive setting. It raised the importance of addressing safety as the primary outcome in the clinical trial proposal that is at the center of this thesis. Last but not least, it showed how neuronavigation gradually improved over time, allowing precise stimulation of deep brain structures and clearly showing how the studies migrated from cortical to deep brain structures such as the thalamus. Therefore, neuronavigation was considered fundamental for modern tFUS research.

As open-source neuronavigation is an upgrowing field, sections 5.2 and 5.3 focused on providing the best practices for open-source neuronavigation that would be used in the proposed protocol. The article provided in section 5.2 demonstrated how a previously described open-source neuronavigation method⁸⁶ presented significant flaws that could be addressed and optimized. It provided not only the practical challenges researchers could find in using such a method but also a complete manual on optimizing it. Although the primary aim was to focus on the proposed clinical trial of this thesis, additional optimizations were suggested even though they were not included in the protocol, such as the simultaneous use of two transducers.

On the other hand, the study proposed in section 5.3 focused on superficial targets that could be useful in tFUS guidance for cortical stimulation based on the International 10-20 system. One more time, the article extrapolated the initial intended objective. It provided evidence for a superficial cortical brain anatomy correlation for five different points instead of only focusing on the one intended to be used (F3, which was highly correlated with the L-DLPFC). The most surprising finding was not connected to the protocol at all: in this study, it was found that the TP3 point was not correlated with any specific cortical region and that neuronavigation is perhaps more important for studies that aim at treating auditory hallucinations than the ones that study the prefrontal cortex.

Finally, with the information gathered in the first study and with the methods described in the second and third studies, it was possible to propose a robust clinical trial in section 5.4. It fulfills many theoretical gaps in the literature, such as the safety of repetitive low-intensity tFUS sessions, the possible use of tFUS for the treatment of patients with TRD, the comparison with a reliable method of cortical neuromodulation and the use of standardized scales to address both safety and efficacy of tFUS in this setting. The article was upgraded several times due to technical advancements and the peer-review process, which provided deep insights into the methodology. Ultimately, it was approved for publication in a highly reputational journal, signaling that the academic community agreed with the proposed methodology.

However, a theoretical clinical trial design would be incomplete without a test of its practical reliability. Therefore, a pilot study was proposed to prove that the method (designed by the articles in sections 5.1, 5.2, and 5.3 was proposed by the article in section 5.4) could be transformed into real practice in a clinical scenario. Moreover, such a pilot provided important practical feedback on details that are frequently neglected in research articles, such as the comfort of the patient and the applicator. These details are considered of utmost importance for a novel technology such as the tFUS, since the objective of transposing its intricate theoretical mechanism into clinical practice requests in-field reliability.

6.1. Contextual analysis of the method

Undeniably, the available resources and the health context must not be neglected when proposing a clinical trial. The Centro de Tecnologia em Medicina

1 Molecular of the Federal University of Minas Gerais (UFMG) possesses two TMS
2 machines, one of which iTBS-suited. It is worth mentioning that TMS is not offered by
3 the Brazilian Public Universal Healthcare System (Sistema Único de Saúde, SUS),
4 making it available to only a few percentage of individuals. Even ECT is considered
5 restricted in SUS, further narrowing the treatment possibilities for such patients.
6 Proposing a clinical trial specifically for patients suffering from TRD would allow them
7 to either be treated with an approved neuromodulation technique for TRD or with a
8 novel technology with enough rationale to stimulate a similar area to that stimulated by
9 the current TMS protocol.

10 Interestingly, this train of thought concludes that a non-inferiority study is best
11 suited for the context in which the current clinical trial is proposed. Although it has pros
12 and cons, as with any study design, it allows the patient to be their own comparator,
13 drastically reducing the sample necessary for achieving a reasonable power. Besides,
14 no sham group is proposed, allowing the patients a 50% chance of being allocated to
15 the TMS group (already approved for treating TRD). Ethically, in this specific time-
16 space context, the non-inferiority design would provide higher access to the general
17 population to a regulatory agency-approved therapeutical method instead of no
18 additional treatment at all. Moreover, this abovementioned chance increases the
19 motivation of the patients to participate in the study, optimizing recruitment efforts. This
20 rationale must not be confused, by any means, with a critique of the use of sham
21 groups, which undoubtedly provide one of the best insights into a given method's
22 efficacy.

23 Although the tFUS has been increasingly studied because of its capacity for
24 deep-brain stimulation, there is still space for cortical stimulation in non-healthy
25 patients. The first justificative is that although cortical low-intensity stimulation seems
26 safe after a single sonication, no study has shown functional and morphological safety
27 after repetitive sessions. Apparently, the point of maximum acoustic energy deposition
28 suffers minimal thermal rise after a single sonication^{41, 85}. However, this must not be
29 extrapolated into repetitive sessions, which are standard in non-invasive
30 neuromodulation techniques. Besides, current open-source neuronavigation methods
31 for low-intensity tFUS stimulation are poorly stable and lack optimization, turning small
32 focus-to-transducer distances into the safest research setting. Considering the
33 justificative contextual rationale, cortical stimulation is more accessible to perform in

practice due to the permissiveness of minor variations across stimulation sessions. This can provide the recently established UFMG neuromodulation team with practical know-how to improve the clinical feasibility and stability necessary for possible deep brain stimulation studies in the future.

6.2. First results and prospections

In contrast with previous studies that used low-intensity tFUS in humans, the protocol proposed by this thesis seemed to be feasible in repetitive sessions in a clinical setting with one patient. Its time was comparable with a standard iTBS TMS session, and no major adverse event with a rational “causal relationship” to the stimulation was reported using the UKU scale. Although the case report failed to improve depressive symptoms as measured in validated scales, it provided significant insight into relevant clinical practices of tFUS sonication. The lack of clinical improvement can be related to many reasons: first of all, the main motivation for reporting this case was to prove and describe a reliable method of tFUS stimulation in a patient with TRD. Safety and effectiveness were secondary outcomes, and, although not effective, the method proved to be safe both functionally and morphologically. Secondly, a single pilot case report does not have the power necessary to establish a non-effectiveness profile, thus the proposal of a robust clinical trial.

Additional explanations can be hypothesized for the lack of clinical response besides the coherent rationale. Differently from TMS, the tFUS stimulates a small area, which may be insufficient to reach clinical response. Moreover, the acoustic intensity proposed for this clinical trial (and, therefore, used in the pilot case) respects all limits set by the FDA, which is non-standard in tFUS research. The reason behind this choice is to first address safety and then, if convenient, efficacy. This train of thought is important since this is the first study to stimulate a specific area of the brain repetitively, in a protocolled scheme.

It is also noteworthy that, besides no clinical improvement was shown, there was evidence of glucose uptake regularization after the sonication protocol. In the case report, the patient presented a baseline PET/CT scan with diffuse low brain uptake of glucose, which was ameliorated immediately after the protocol. This may be explained, in part, by the dense circuitry involving the DLPFC in the physiopathology of depression¹³², meaning that the stimulation of a single cortical area can lead to

neuromodulation in different regions of the brain. The gap between the clinical and neuroradiological results is still up for debate, but it can be explained by the multifaceted pathophysiology of depression and hopefully will be addressed with more robustness in the proposed clinical trial.

It is important to mention that, in practice, staying in the same position for almost one hour may be uncomfortable. This pilot was important to determine minor details that could maximize the patient's comfort and, therefore, reduce the dropout rate during the clinical trial. A noteworthy example is the choice of listening or not to music while waiting or during sonications, which the patient did not choose. Other details comprise the position of the head during the sonication, the comfort of both the patient and the applicator, the need for stabilization using the hands, and the best practices to minimize time in the registration procedure needed for neuronavigation.

6.3. The contribution of practical methodological studies to the science

The so-called publication bias is well established in both medical¹⁷⁷ and non-medical research, although it is more prominent in biomedical sciences¹⁷⁸. The term refers to a phenomenon of scientific publication favoring positive results, while negative results are commonly unpublished or become less cited¹⁷⁹. Although the term “negative results” can be commonly attributed to statistically non-relevant studies, it also includes manuscripts with truly inconclusive results (e.g., the number of participants is too low), well-conducted studies with clear evidence of no effect, or papers evidencing harm when benefits were expected¹⁸⁰. Recent data indicates that publication bias still plays a significant role in science, in despite of a growing initiative to support the publication of negative results^{180, 181}. It is estimated that around 85.9% of all accepted manuscripts have positive results, in contrast with a previous proportion of 70% in the 90s¹⁸². Moreover, around 50% of all negative results are presumably unpublished at all¹⁸³.

In the gray area between negative results and “positive results” lie methodological manuscripts, which are increasingly losing space in the scientific environment¹⁸⁴. They furnish insights into research planning, execution, analysis, and documentation. The debate about how to improve methodology articles dates back to at least two decades ago¹⁸⁵, and they seem to struggle progressively more in being published¹⁸⁴. Moreover, there is a consensus that only one third of papers describe satisfactorily their methods¹⁸⁶⁻¹⁸⁸, which could compromise the studies' results in about

17% of the cases¹⁸⁸. Although these data are frequently ignored by the scientific community, Dr John P. A. Ioannidis, MD, PhD, claimed in 2005 that “most published research findings are false”¹⁸⁹, in an article debated over 7,500 articles and viewed more than 3 million times to this date.

Among the explicit and implicit consequences of publication bias, the impact on replication is considerably relevant^{190, 191}. It is presumably more important in novel technologies, especially in the early stages of clinical trials, during which positive results warrant further studies in the field. On the other hand, albeit with considerable disadvantages against positive results articles, negative results are relatively feasible for publication when they rely on already established interventions (e.g., the use of Esketamine for treatment-resistant depression¹⁹²). In the latter case, the study establishes limits for previous generalized data about the specific subject.

The combination of publication bias, unsatisfactory methodology description, and the prevalence of negative results’ publication in later clinical trials shows how challenging the research with novel technologies can be. In an experimental setting, challenges are expected, and turning theory into practice often requires gathering pieces of different predecessors together. Furthermore, high variability is expected at early stages of research due to variations between multiple centers and personal interpretations, leading to a necessity of standardize and justify specific methods and protocols.

In the opposite direction of the abovementioned inference, this thesis focuses mostly on the practical aspects of a promising neuromodulation technique, which seems to be a considerable literature gap. Most of the workload was presented descriptively; the clinical trial is only proposed by this thesis, and a single case report is presented. However, it is still worth mentioning that studies that detail procedures, failures, and descriptive attempts can be extraordinarily useful for future researchers, although this postulate is rarely published or written. It does not mean to imply, by any means, that positive results should be discouraged, being a mere statement of how different types of research can converge to the progress of science.

6.4. Limitations

This thesis has a considerable number of limitations, most of them of special relevance. This raises the concern that the results must be carefully interpreted and

1 that ameliorations are strongly recommended, although the author considers that the
2 workload employed in this study may help future researchers. Part of the limitations
3 were already discussed in session 6.3. since antitheses were raised and considered
4 in the process of synthesis.

5 The first and probably most worrisome of this thesis is its reproducibility. As
6 mentioned in section 2, the clinical trial proposed (and, as a consequence, the pilot
7 study described) takes into consideration the resources of a given center, in a specific
8 momentum of tFUS research in humans. Although efforts were made to maximize
9 reproducibility (e.g., focusing on an open-source neuronavigation method), it is
10 undeniable that minimal hardware, software, and human resources are needed for
11 reproduction. They can be exemplified by (but not limited to) the availability of 3T
12 morphological MRI and PET/CT images, the need of an infrared-based optical camera
13 for stereotaxic neuronavigation, fiducial markers, a focused ultrasound transducer, and
14 specific material. This last resource, as mentioned in section 6.2., can be considered
15 as “unique”, and therefore there is a strong need for maintenance and backup material.

16 Although the section 5.2. provides an optimized method for open-source
17 neuronavigation, it is undeniable that its practical usage depends on training and may
18 have a harsh learning curve. This need of training the involved personnel can be
19 extended to many parts of the thesis, such as preparing the images before the
20 sonication, training researchers to perform validated scales, and following strictly the
21 manual provided in Supplementary File 2. This limitation is a strong one, since the core
22 of this thesis is to provide a “clinically feasible method” for low-intensity tFUS sonication
23 in both research and clinical practices. However, given the state-of-the-art of low-
24 intensity tFUS research so far, maybe such easy learning curve is still unreliable.

25 Another limitation is the strong antithesis that the number of applicators in the
26 study presented in section 5.3. is not enough to state inter-applicator concordance. For
27 this thesis to be considered, further studies are recommended. Also, although the
28 protocol presented in section 5.4. preconizes a 2-month follow-up of the patients during
29 the clinical trial, this follow-up was not included in the case report presented in section
30 5.6. This is a considerable limitation, since the results presented (especially the
31 positive ones) may be absent in the follow-up session, which would contradict the
32 hypothesis that repetitive tFUS sonications could provide long-term clinical outcomes.

Additional limitations of this thesis can be listed. Firstly, few centers have already tested the optimized neuronavigation method, which may be unreliable in other hardware or settings. Secondly, a single patient in the pilot study provides only a case report, from which few conclusions and insights can be taken. Thirdly, most of the hardware used to process images, neuronavigate, and prepare the virtual scene for sonications can be considered “potent” due to federal and local financial support provided for this study. Such potent hardware may not be present in other centers, supporting the antithesis that clinical reliability may be compromised by the focus of this thesis in a single center. Additionally, it is an informal consensus that open-source software can be non-user-friendly, which is the case of most of the software used in the results of this thesis. Personnel training and user-friendliness is still a challenge in practical tFUS sonication, in both research and clinical settings, and additional studies are needed to optimize this characteristic.

The proposed clinical trial is also not free of limitations. Due to contextual resources, it does not include neurophysiological measurements, such as functional MRI and EEG, which could be useful in evaluating the patients. Although the UKU scale was carefully selected due to its broad range of potential adverse events, there is no specific scale for the tFUS. Therefore, the safety measurements of the study may lack relevant data that is still unknown or is not standardized in particular questionnaires. Besides, since this is the first proposed repetitive protocol using the tFUS, there will be no way of comparing its results with other protocols (e.g., traditional TMS protocols instead of iTBS). Finally, the protocol does not establish if sound will be used during the sonications, so this decision is outsourced to the patient's comfort. Since the protocol deals with a novel technology that uses ultrasonic waves aiming at neuromodulation, this might be a confusion factor and must be noted in the final analysis of the clinical trial.

7. CONCLUSIONS

This work provides a ground for future research with low-intensity tFUS in humans by addressing the gaps in the literature so far, optimizing the methods that can be used for practical neuromodulation, and proposing a robust clinical trial that uses this methodology. Moreover, it tested the proposed method in a pilot study that

1 demonstrated its clinical feasibility, the safety of multiple sonications in a patient with
2 TRD, and the tolerability of such protocol when applied to the L-DLPFC. Although most
3 of the work is theoretical, since it needs to provide the rationale for proposing a clinical
4 trial, the practical aspects of low-intensity sonication were described in detail to best
5 contribute to future tFUS studies in humans.

6 The practical aspects of this thesis proved that the neuronavigation process
7 proposed in section 5.2. is reliable and easier to use than the previous available one,
8 as tested in two centers. It may be considered, therefore, accurate and reproducible.
9 The L-DLPFC was successfully achieved in a computational method. The whole pre-
10 procedure preparation could be done before the patient enters the stimulation site,
11 minimizing the time needed for registration (a fundamental aspect of neuronavigation).
12 So far, the pilot case report supported the hypothesis that this repetitive protocol is safe
13 and tolerable, and this thesis provides a proposal of a future clinical trial that has the
14 possibility of evaluating both the safety and efficacy of low-intensity tFUS sonication in
15 patients with TRD. Nevertheless, there is still a need of replication of these methods
16 by different centers, as well as the assessment of safety and efficacy.

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13

1 9. SUPPLEMENTARY MATERIAL

2 9.1. Photos of the evolution of hardware use in this thesis

3 9.1.1. NDI Polaris Vicra used in neuronavigation

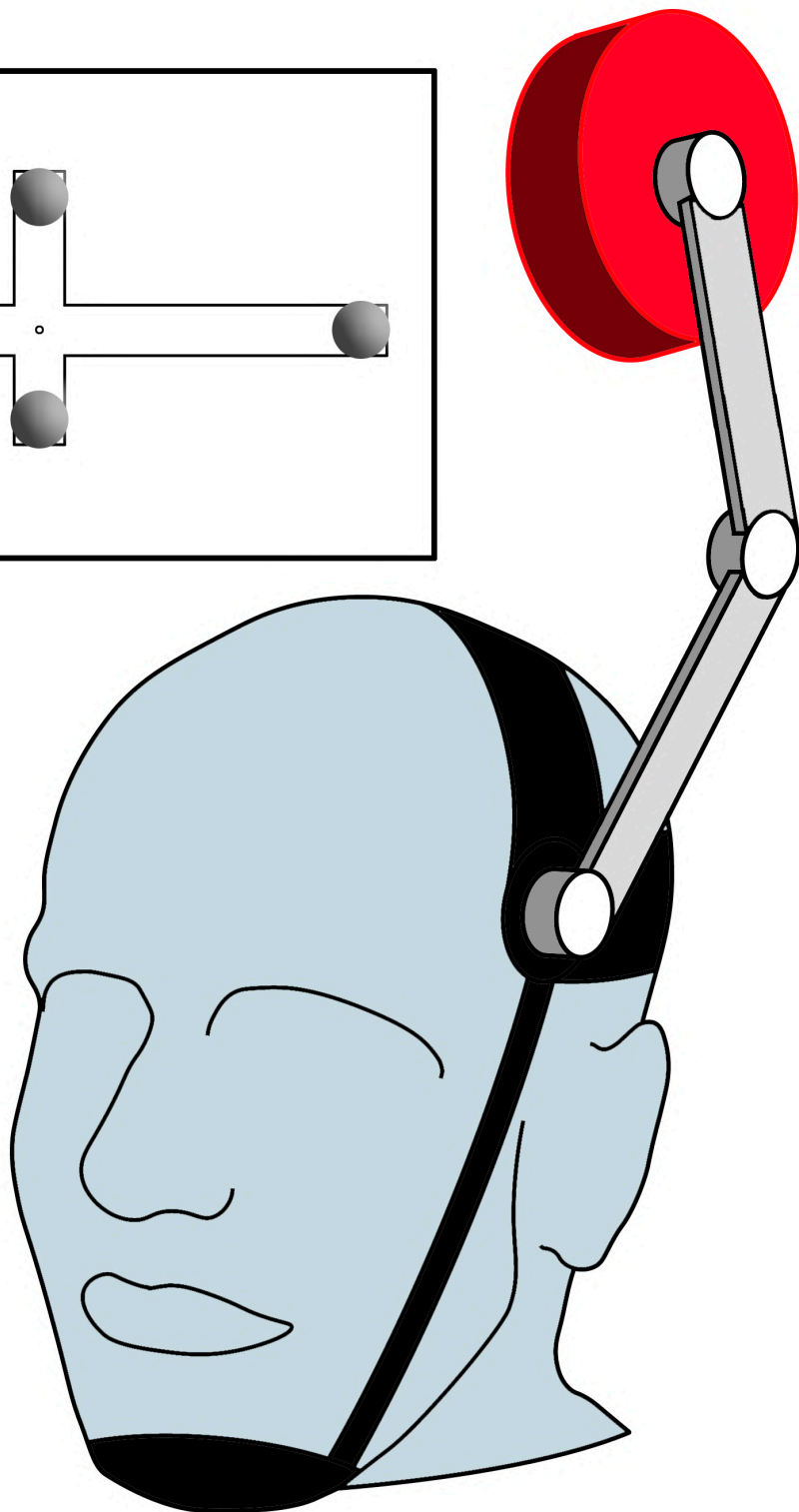
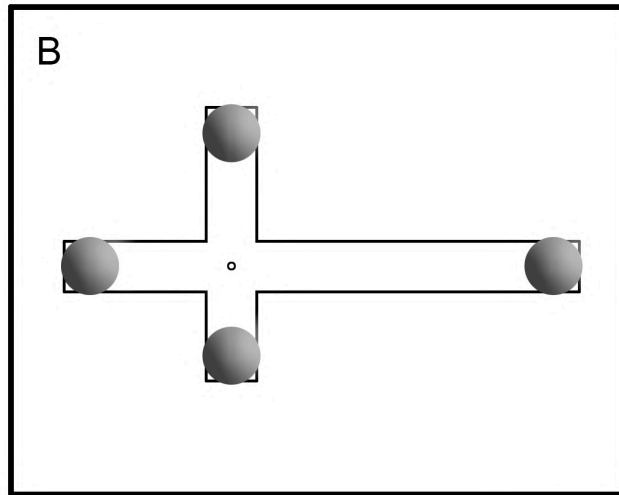


- 1 9.1.2. First model of a helmet built to hold the fidutials and the transducer to the
- 2 patient's head.

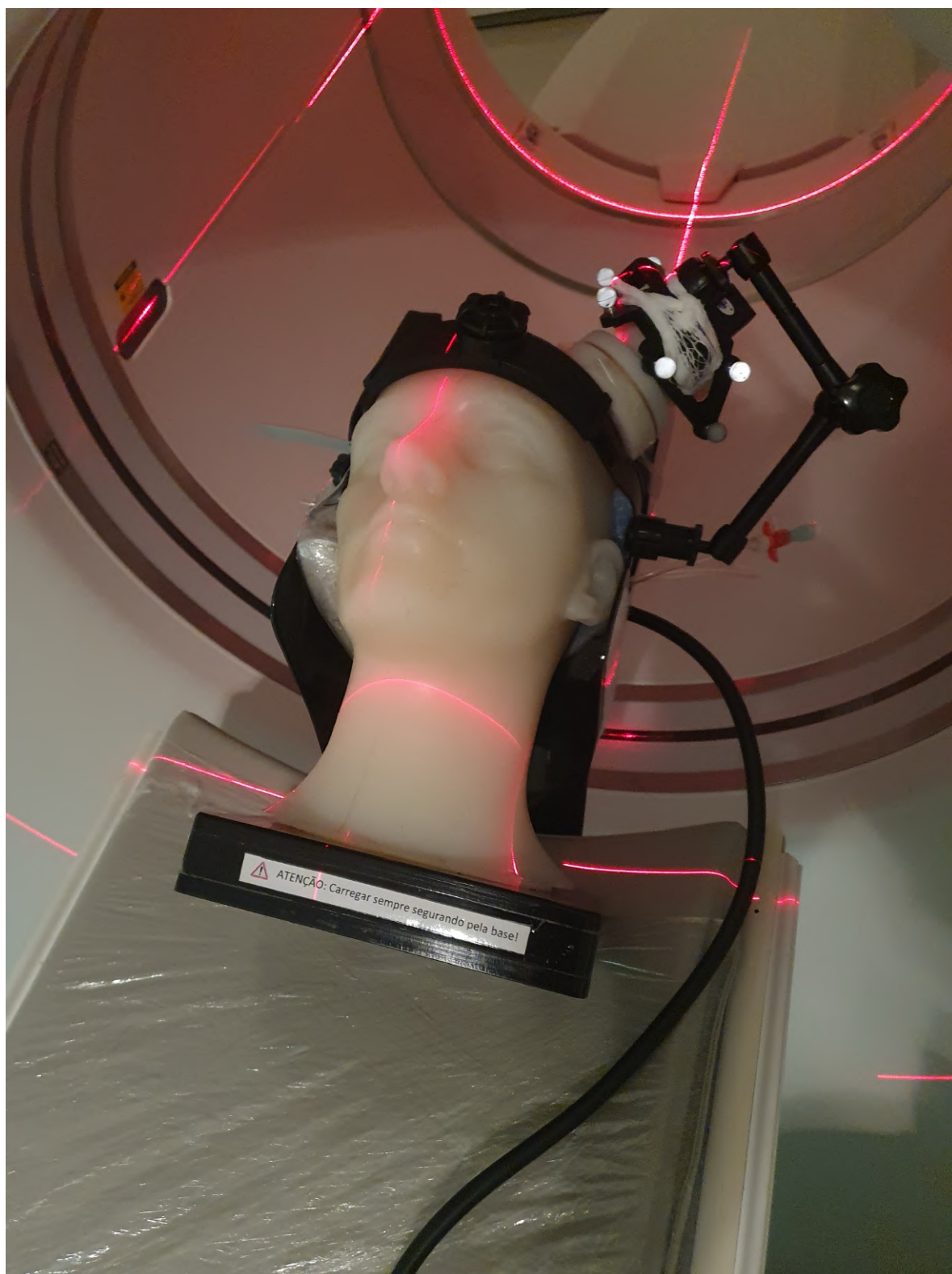


- 1 9.1.3. Illustration of a proposed helmet to hold the fiducials and the transducer
- 2 to the patient's head.

A



- 1 9.1.4. Photo taken of the helmet in a computerized tomography scan to evaluate
- 2 feasibility of sonications inside of the machine.

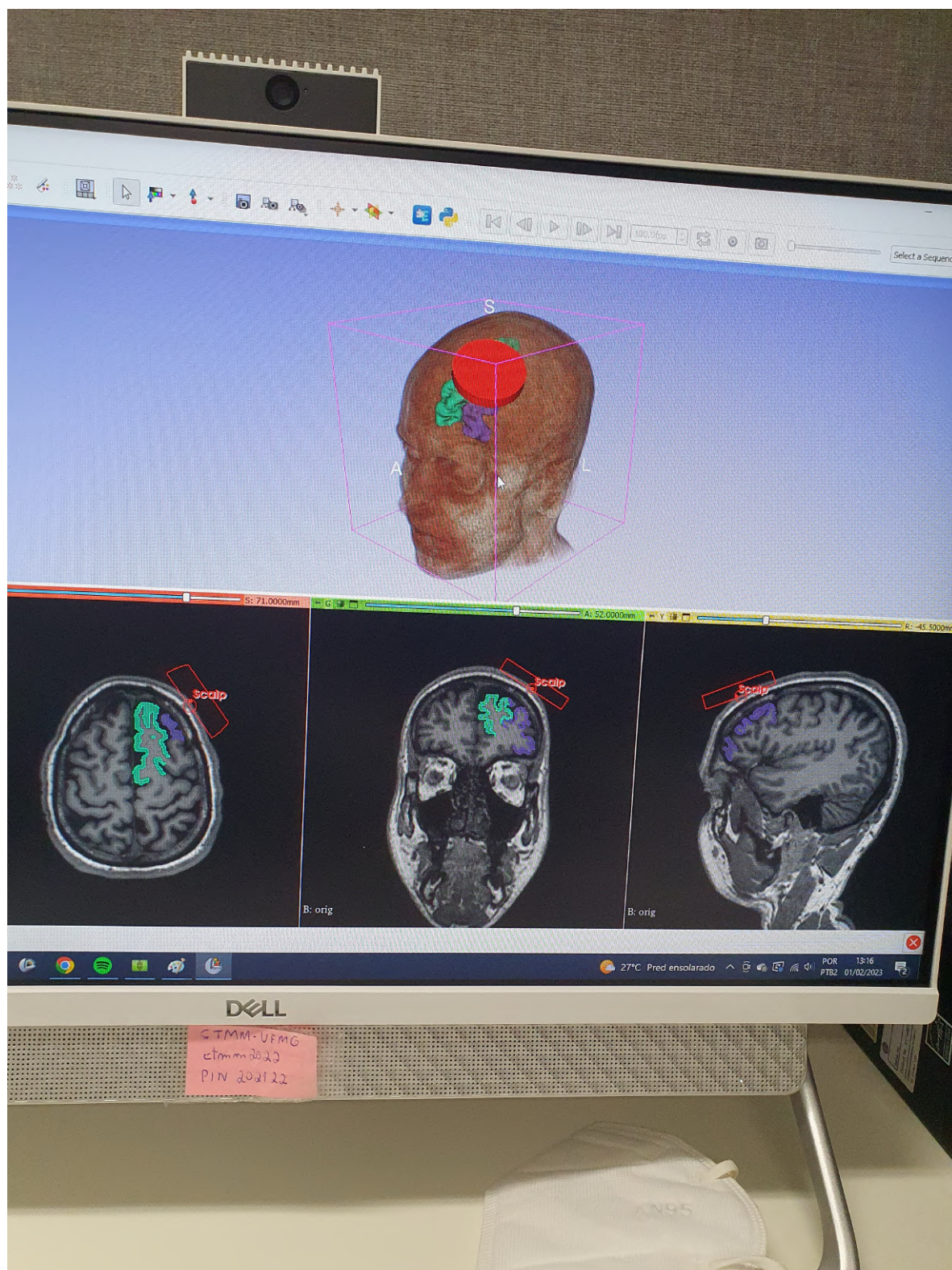


1 **9.1.5. Neuronavigation test using the helmet in a human head model.**

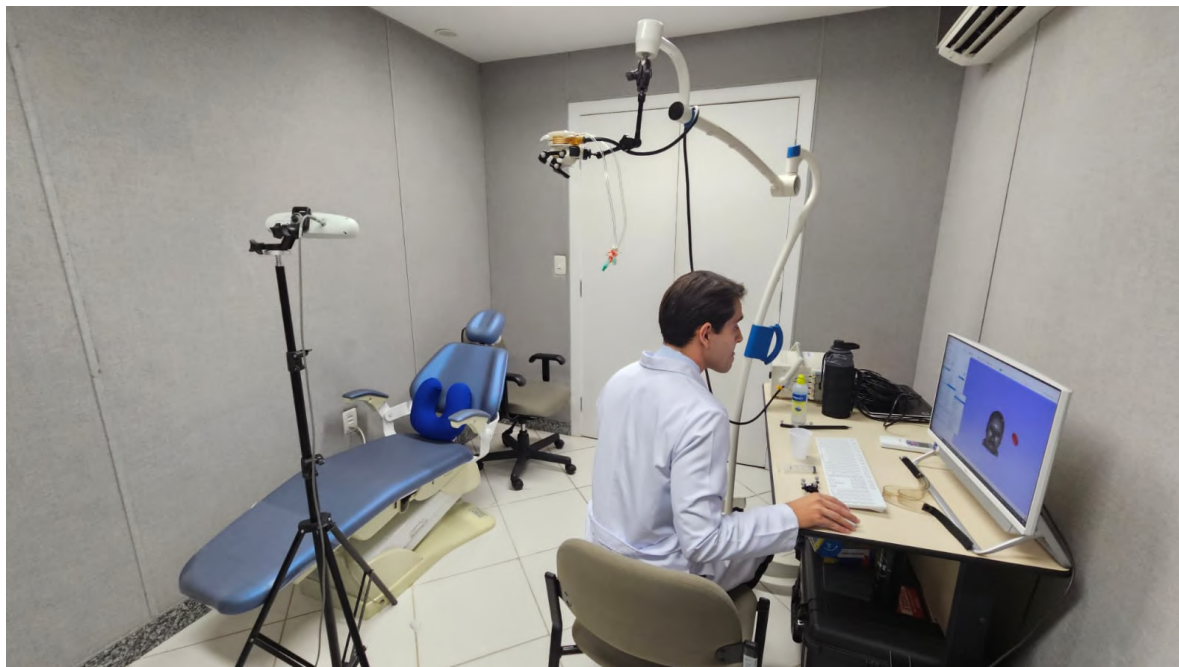


2

1 9.1.6. Neuronavigation test using a human volunteer.



- 1 9.1.7. Experimental setup of a different chair for neuromodulation, using an
2 external holder to the transducer, after the conclusion that the helmet was not
3 clinically feasible.



- 4
5 9.1.8. Current holder of the transducer attached to the chair.



- 6
7
8 9.2. Institutional ethical report (in Brazilian Portuguese)

UNIVERSIDADE FEDERAL DE
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PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: USO DO ULTRASSOM FOCALIZADO TRANSCRANIANO NO TRATAMENTO DA DEPRESSÃO RESISTENTE

Pesquisador: Marco Aurélio RomanoSilva

Área Temática: Equipamentos e dispositivos terapêuticos, novos ou não registrados no País;

Versão: 5

CAAE: 56451022.1.0000.5149

Instituição Proponente: Faculdade de Medicina da UFMG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.266.375

Apresentação do Projeto:

A emenda tem como objetivo efetivar alteração do cronograma de pesquisa, considerando a sobreposição de projetos que utilizam a estimulação transcraniana no Centro de Tecnologia em Medicina Molecular (CTMM), dessa forma, são propostas as seguintes mudanças: 1) Antecipar a realização da estimulação transcraniana de 01/12/2023 para 02/10/2023, tendo em vista que o aparelho de Ultrassom Focalizado já está pronto para uso no CTMM; 2) Prorrogar o prazo final para a realização da estimulação transcraniana de 30/04/2024 para 30/10/2026, considerando que outras pesquisas com o aparelho de TMS estão aprovadas, havendo sobreposição de horários; 3) Prorrogar o prazo de recrutamento dos participantes para 01/10/2026 de forma a alinhar ao novo prazo para a realização da estimulação transcraniana, no entanto, o início do recrutamento será mantido para 31/08/2023; 4) Prorrogar o prazo final do projeto de 30/09/2024 para 31/12/2026, tendo em vista a prorrogação das etapas de recrutamento e de estimulação transcraniana.

Objetivo da Pesquisa:

Objetivo Primário:

Analisar a eficácia e a segurança do uso repetitivo do ultrassom focalizado transcraniano (tFUS – transcranial focused ultrasound) no tratamento da depressão resistente ao tratamento (DRT), em comparação com uma alternativa já estudada e validada para esse tratamento.

Endereço: Av. Presidente Antonio Carlos, 6627 2º. Andar Sala 2005 Campus Pampulha

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Continuação do Parecer: 6.266.375

Objetivo Secundário:

- Avaliar a tolerabilidade e os efeitos colaterais do uso repetitivo do tFUS em indivíduos com DRT.
- Avaliar modificações neuroestruturais e neurofuncionais após o uso do tFUS em indivíduos com DRT.
- Avaliar se fatores clínicos modificáveis e não modificáveis (como sexo, idade, etnia ou farmacoterapia) estão associados ao efeito terapêutico do tFUS.
- Avaliar diferenças de eficácia entre o tratamento com o tFUS e o tratamento com a estimulação magnética transcraniana.

Avaliação dos Riscos e Benefícios:

Como não há mudança nos métodos e critérios de inclusão e alteração, os riscos estão inalterados em relação à versão atual do projeto aprovado.

Comentários e Considerações sobre a Pesquisa:

Inalterados em relação ao projeto mais recente aprovado.

Considerações sobre os Termos de apresentação obrigatória:

Os termos utilizados estão adequados e documentos anteriormente apresentados encontram-se válidos.

Recomendações:

Sem recomendações.

Conclusões ou Pendências e Lista de Inadequações:

N/A

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente (Resolução CNS 466/12), o CEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

O presente projeto, seguiu nesta data para análise da CONEP e só tem o seu início autorizado após a aprovação pela mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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Continuação do Parecer: 6.266.375

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_2185308_E2.pdf	25/07/2023 15:53:26		Aceito
Cronograma	Cronograma_Revisado.pdf	25/07/2023 15:52:01	BERNARDO DE MATTOS VIANA	Aceito
Outros	Justificativa_emenda.pdf	25/07/2023 15:51:51	BERNARDO DE MATTOS VIANA	Aceito
Outros	ABEP.pdf	30/05/2023 17:19:39	Gustavo dos Santos Alves Maria	Aceito
Outros	QFC.pdf	30/05/2023 17:19:20	Gustavo dos Santos Alves Maria	Aceito
Outros	QIDS_SR16.pdf	30/05/2023 17:18:59	Gustavo dos Santos Alves Maria	Aceito
Outros	DGI.pdf	30/05/2023 17:18:33	Gustavo dos Santos Alves Maria	Aceito
Outros	WHOQoL_Bref.pdf	30/05/2023 17:18:10	Gustavo dos Santos Alves Maria	Aceito
Outros	WHODAS_20.pdf	30/05/2023 17:17:42	Gustavo dos Santos Alves Maria	Aceito
Outros	ESV.pdf	30/05/2023 17:16:45	Gustavo dos Santos Alves Maria	Aceito
Outros	Escala_de_Efeitos_Colaterais_de_Udvalg.pdf	30/05/2023 17:16:25	Gustavo dos Santos Alves Maria	Aceito
Outros	MADRS.pdf	30/05/2023 17:15:55	Gustavo dos Santos Alves Maria	Aceito
Outros	PANAS.pdf	30/05/2023 17:15:27	Gustavo dos Santos Alves Maria	Aceito
Outros	HAMD.pdf	30/05/2023 17:15:01	Gustavo dos Santos Alves Maria	Aceito
Outros	Carta_resposta.docx	30/05/2023 17:13:45	Gustavo dos Santos Alves Maria	Aceito
Outros	Carta_respostaassinada.pdf	30/05/2023 17:12:46	Gustavo dos Santos Alves Maria	Aceito
Outros	Carta.docx	27/03/2023 08:32:39	Gustavo dos Santos Alves Maria	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	27/03/2023 08:26:02	Gustavo dos Santos Alves Maria	Aceito
Projeto Detalhado / Brochura Investigador	u180p1056_6213aad188912_Projetodepesquisa.pdf	07/03/2022 11:30:34	Marco Aurélio RomanoSilva	Aceito
Outros	ParecerSAMM002_2022.pdf	07/03/2022 11:22:37	Marco Aurélio RomanoSilva	Aceito
Declaração de Instituição e Infraestrutura	CTMM_anuencia.pdf	07/03/2022 11:19:28	Marco Aurélio RomanoSilva	Aceito

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Continuação do Parecer: 6.266.375

Folha de Rosto	FR_tFUS.pdf	07/03/2022 11:08:38	Marco Aurélio RomanoSilva	Aceito
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Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Sim

BELO HORIZONTE, 28 de Agosto de 2023

Assinado por:
Corinne Davis Rodrigues
(Coordenador(a))

Endereço: Av. Presidente Antonio Carlos, 6627 2º. Andar Sala 2005 Campus Pampulha
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Página 04 de 04

- 1
- 2 9.3. Main scales used in the clinical assessment of depressive symptoms and adverse
- 3 events.

1 9.3.1. Self-reported Quick Inventory of Depressive Symptomatology

QIDS-SR16

Page 1

1) Nome completo _____

2) Data _____

Por favor, assinale em cada item a resposta que melhor o(a) descreve nos últimos sete dias.

- 3) 1. Pegar no sono
- ☐ Levo pelo menos 30 minutos para pegar no sono, mais da metade das vezes (4 dias ou mais nos últimos 7 dias)
 - ☐ Nunca levo mais do que 30 minutos para pegar no sono
 - ☐ Levo pelo menos 30 minutos para pegar no sono, menos da metade das vezes (3 dias ou menos nos últimos 7 dias)
 - ☐ Levo mais de uma hora para pegar no sono, mais da metade das vezes (4 dias ou mais nos últimos 7 dias)
-
- 4) 2. Despertar durante o sono
- ☐ Não acordo durante a noite
 - ☐ Tenho um sono leve e agitado, acordando por períodos curtos em cada noite
 - ☐ Acordo pelo menos uma vez por noite, mas volto a dormir com facilidade
 - ☐ Acordo mais do que uma vez por noite e fico acordado(a) por 20 minutos ou mais, mais da metade das vezes (4 dias ou mais nos últimos 7 dias)
-
- 5) 3. Acordar Cedo Demais
- ☐ Na maioria das vezes acordo no horário em que preciso, ou no máximo até 30 minutos antes
 - ☐ Mais da metade das vezes (4 dias ou mais nos últimos 7 dias), acordo mais de 30 minutos antes do que preciso para levantar
 - ☐ Quase sempre acordo pelo menos em torno de uma hora antes da hora que eu preciso, mas acabo voltando a dormir
 - ☐ Acordo pelo menos uma hora antes do que preciso e não consigo voltar a dormir
-
- 6) 4. Dormir demais
- ☐ Durmo até 7-8 horas por noite, sem cochilos durante o dia
 - ☐ Durmo até 10 horas num período de 24 horas, incluindo cochilos
 - ☐ Durmo até 12 horas num período de 24 horas, incluindo cochilos
 - ☐ Durmo mais do que 12 horas num período de 24 horas, incluindo cochilos
-
- 7) 5. Sentir-se triste
- ☐ Não me sinto triste
 - ☐ Sinto-me triste menos da metade das vezes (3 dias ou menos nos últimos 7 dias)
 - ☐ Sinto-me triste mais da metade das vezes (4 dias ou mais nos últimos 7 dias)
 - ☐ Sinto-me triste quase o tempo todo

Favor responder 6 ou 7

- 8) 6. Diminuição de apetite
- ☐ Meu apetite habitual não mudou
 - ☐ Como com menor frequência ou menores quantidades de comida que o habitual
 - ☐ Como muito menos que o habitual e só com um esforço pessoal
 - ☐ Como raramente num período de 24 horas e só com um esforço pessoal extremo ou quando outros me convencem a comer
-
- 9) 7. Aumento de apetite
- ☐ Meu apetite habitual não mudou
 - ☐ Sinto necessidade de comer mais frequentemente do que o habitual
 - ☐ Regularmente, como com mais frequência e/ou em maiores quantidades do que o habitual
 - ☐ Sinto-me compelido a comer demais tanto nas refeições como entre elas

Favor responder 8 ou 9

- 10) 8. Aumento de Peso (Nas Duas Últimas Semanas)
- ☐ Não tive qualquer mudança no meu peso
 - ☐ Sinto-me como se tivesse tido um pequeno aumento de peso
 - ☐ Engordei 1 quilo ou mais
 - ☐ Engordei 2,5 quilos ou mais
-
- 11) 9. Perda de Peso (Nas Duas Últimas Semanas)
- ☐ Não tive qualquer mudança no meu peso
 - ☐ Sinto-me como se tivesse tido uma pequena perda de peso
 - ☐ Perdi 1 quilo ou mais
 - ☐ Perdi 2,5 quilos ou mais
-
- 12) 10. Concentração e Tomada de Decisões
- ☐ Não houve mudança na minha capacidade habitual de me concentrar ou de tomar decisões
 - ☐ Ocasionalmente me sinto indeciso(a) ou acho que minha atenção flutua
 - ☐ Na maior parte do tempo, me esforço para prestar atenção ou para tomar decisões
 - ☐ Não consigo me concentrar o suficiente para ler ou não consigo tomar nem mesmo decisões pequenas
-
- 13) 11. Visão de Mim Mesmo
- ☐ Considero-me tão valioso(a) e merecedor(a) como os outros
 - ☐ Recrimino-me mais do que o habitual
 - ☐ Acredito muito que causo problemas para os outros
 - ☐ Penso quase constantemente sobre pequenos e grandes defeitos em mim mesmo(a)
-
- 14) 12. Idéias de Morte e Suicídio
- ☐ Não penso em suicídio ou morte
 - ☐ Sinto que a vida é vazia e me pergunto se vale a pena viver
 - ☐ Penso em suicídio ou morte várias vezes por semana durante vários minutos
 - ☐ Penso em suicídio ou morte várias vezes por dia com algum detalhe, ou fiz planos específicos de suicídio, ou realmente tentei me matar

-
- 15) 13. Interesse Geral
- ☐ Não houve mudanças do habitual quanto ao interesse que tenho em outras pessoas e atividades
 - ☐ Noto que estou menos interessado(a) em pessoas ou em atividades
 - ☐ Acho que tenho interesse em apenas uma ou duas das atividades a que me dedicava anteriormente
 - ☐ Não tenho praticamente interesse nenhum nas atividades a que me dedicava anteriormente
-
- 16) 14. Nível de Energia
- ☐ Não há mudanças no meu nível habitual de energia
 - ☐ Fico cansado(a) mais facilmente do que o habitual
 - ☐ Tenho que fazer um grande esforço para começar ou acabar minhas atividades diárias habituais (por exemplo, fazer compras, lição de casa, cozinhar ou trabalhar)
 - ☐ Eu não consigo realmente desempenhar a maioria das minhas atividades diárias, porque simplesmente não tenho energia
-
- 17) 15. Sentimento de lentidão
- ☐ Penso, falo e me movimento na minha velocidade habitual
 - ☐ Acho que o meu pensamento está mais lento ou a minha voz parece sem brilho ou monótona
 - ☐ Levo vários segundos para responder à maioria das perguntas e tenho certeza que o meu pensamento está lento
 - ☐ Sinto-me frequentemente incapaz de responder a perguntas sem um esforço extremo
-
- 18) 16. Sentir-se Agitado
- ☐ Não me sinto agitado(a)
 - ☐ Fico frequentemente irrequieto(a), torcendo as mãos ou precisando trocar a posição em que estou sentado(a)
 - ☐ Tenho impulsos de me movimentar e estou bastante agitado(a)
 - ☐ Há ocasiões em que fico incapaz de permanecer sentado(a) e preciso andar de um lado para o outro
-

1 9.3.2. Hamilton Scale for Depression (only first 17 questions used)

Escala de Avaliação de Depressão de Hamilton (HAM-D) ^{Page 1}

1)	Nome completo	
2)	Data	
3)	AVALIADOR: Preencher de acordo com critérios clínicos.	
4)	Humor depressivo (tristeza, desesperança, desamparo, inutilidade)	☐ Ausente ☐ Sentimentos relatados somente se perguntados ☐ Sentimentos relatados espontaneamente, com palavras ☐ Comunica os sentimentos não com palavras, mas com expressão facial, postura, voz e tendência ao choro ☐ O paciente comunica quase que exclusivamente esses sentimentos, tanto em seu relato verbal como na comunicação não-verbal
5)	Sentimentos de culpa:	☐ Ausente ☐ Auto-recriminação, acha que decepcionou outras pessoas ☐ Ideias de culpa ou ruminções de erros ou ações pecaminosas (más) no passado ☐ Paciente acha que a doença atual é uma punição (castigo). Delírio de culpa ☐ Ouve vozes que o acusam ou denunciam e/ou tem alucinações visuais ameaçadoras
6)	Suicídio:	☐ Ausente ☐ Acha que não vale a pena viver ☐ Deseja estar morto ou pensa em uma possível morte para si ☐ Ideias ou atitudes suicidas ☐ Tentativas de suicídio
7)	Insônia inicial:	☐ Sem dificuldades para iniciar o sono ☐ Queixa de dificuldade ocasional para iniciar o sono, ou seja, mais que meia hora ☐ Queixa de dificuldade para iniciar o sono todas as noites
8)	Insônia intermediária:	☐ Sem dificuldade ☐ Queixa de agitação e perturbação durante a noite ☐ Acorda durante a noite - qualquer saída da cama (exceto por motivos de necessidade fisiológica)
9)	Insônia tardia:	☐ Sem dificuldade ☐ Acorda durante a madrugada, mas volta a dormir ☐ Não consegue voltar a dormir se levantar da cama durante a noite

-
- 10) Trabalho e atividades:
- ☐ Sem dificuldades
 - ☐ Pensamentos e sentimentos de incapacidade, fadiga ou fraqueza, relacionados a atividades, trabalho ou passatempos
 - ☐ Perda de interesse em atividades, passatempos ou trabalho, quer relatado diretamente pelo paciente, quer indiretamente por desatenção, indecisão ou vacilação (sente que precisa se esforçar para o trabalho ou outras atividades)
 - ☐ Diminuição no tempo gasto em atividades ou queda de produtividade. No hospital, o paciente ocupa-se por menos de três horas por dia em atividades (trabalho hospitalar ou passatempos) com exceção das tarefas rotineiras da enfermagem
 - ☐ Parou de trabalhar devido à doença atual. No hospital, sem atividades, com exceção das tarefas rotineiras da enfermagem, ou se não consegue realizá-las sem ajuda
-
- 11) Retardo (lentificação do pensamento e da fala, dificuldade de concentração, diminuição da atividade motora):
- ☐ Pensamentos e fala normais
 - ☐ Lentificação discreta à entrevista
 - ☐ Lentificação óbvia durante a entrevista
 - ☐ Entrevista difícil
 - ☐ Estupor completo
-
- 12) Agitação:
- ☐ Nenhuma
 - ☐ Inquietação
 - ☐ Mexe as mãos, cabelos etc.
 - ☐ Movimenta-se bastante, não consegue permanecer sentado durante a entrevista
 - ☐ Retorce as mãos, rói as unhas, puxa os cabelos, morde os lábios
-
- 13) Ansiedade psíquica:
- ☐ Sem dificuldade
 - ☐ Tensão e irritabilidade subjetivas
 - ☐ Preocupa-se com trivialidades
 - ☐ Atitude apreensiva aparente no rosto ou na fala
 - ☐ Paciente expressa medo sem ser perguntado
-
- 14) Ansiedade - somática:
- ☐ Ausente
 - ☐ Duvidoso ou trivial: sintomas menores, relatados quando questionados
 - ☐ Leve: paciente descreve espontaneamente os sintomas, que não são acentuados ou incapacitantes
 - ☐ Moderado: mais do que 2 sintomas e com maior frequência. São acompanhados de estresse subjetivo e prejudicam o funcionamento normal
 - ☐ Grave: numerosos sintomas, persistentes e incapacitantes na maior parte do tempo, ou ataques de pânico quase diariamente
-
- 15) Sintomas gastrointestinais - somáticos:
- ☐ Nenhum
 - ☐ Perda de apetite, mas come sem necessidade de insistência
 - ☐ Dificuldade para comer se não insistirem

- | | |
|--|---|
| 16) Sintomas somáticos gerais: | ☐ Nenhum
☐ Peso em membros, costas ou cabeça; dor nas costas, na cabeça ou nos músculos. Perda de energia e fadigabilidade
☐ Qualquer sintoma bem caracterizado e nítido |
| 17) Sintomas Genitais - (como perda de libido, distúrbios menstruais): | ☐ Ausentes
☐ Leves ou infrequentes: perda de libido, desempenho sexual prejudicado
☐ Óbvio e graves: perda completa do interesse sexual |
| 18) Hipocondria: | ☐ Ausente
☐ Auto-observação aumentada (com relação ao corpo)
☐ Preocupação com a saúde
☐ Queixas frequentes, pedidos de ajuda etc.
☐ Delírios hipocondríacos |
| 19) Perda de Peso (desde o início da doença ou da última avaliação) | ☐ Sem perda de peso ou perda de peso NÃO causada pela doença atual
☐ Perda de peso provavelmente causada pela doença atual. Perda de menos de meio quilo
☐ Perda de peso definitivamente causada pela doença atual. Perda de meio quilo ou mais |
| 20) Crítica (Consequência da doença): | ☐ Reconhece estar deprimido e doente OU não estar deprimido no momento
☐ Reconhece estar, mas atribui a causa à má alimentação, ao clima, ao excesso de trabalho, a um vírus, à necessidade de descanso etc.
☐ Nega estar doente |
| 21) Nesta última semana você se sentiu melhor ou pior em algum período específico do dia - manhã ou noite? | ☐ Nenhuma
☐ Leve
☐ Grave |
| 22) Anote se os sintomas são piores de manhã ou à noite. SE NÃO HOUVER variação diurna, marque nenhuma: | ☐ Sem variação ou não deprimido no momento
☐ Pior pela manhã
☐ Pior a tarde/à noite |
| 23) Quando presente, anote a gravidade da variação: | ☐ Nenhuma
☐ Leve
☐ Grave |
| 24) Despersonalização e Desrealização (como sensação de irrealidade a idéias niilistas) | ☐ Ausentes
☐ Leves
☐ Moderadas
☐ Graves
☐ Incapacitantes |
| 25) Sintomas Paranóides: | ☐ Nenhum
☐ Desconfiado
☐ Idéias de referência
☐ Delírios de referência e perseguição |
| 26) Sintomas Obsessivos e Compulsivos: | ☐ Nenhum
☐ Leves
☐ Graves |

Hamilton, M: A rating scale for depression, Journal of Neurology, Neurosurgery, and Psychiatry 23:56-62, 1960

1 9.3.3. Montgomery-Åsberg Depression Rating Scale

Escala de Avaliação para Depressão de Montgomery & Åsberg (MADRS)

Page 1

1) Nome completo _____

2) Data _____

AVALIADOR: Preencher de acordo com critérios clínicos.

- | | | |
|----|--|---|
| 3) | 1. Tristeza Aparente

Representando desânimo, tristeza e desespero (mais que um abatimento simples e transitório), refletidos na fala, expressão facial a postura. Avalie pela profundidade e incapacidade de alegrar-se. | ☐ 0. Nenhuma tristeza
☐ 1.
☐ 2. Parece abatido, mas se alegra sem dificuldades.
☐ 3.
☐ 4. Parece triste e infeliz a maior parte do tempo.
☐ 5.
☐ 6. Parece muito triste todo o tempo. Extremamente desanimado. |
| 4) | 2. Tristeza Relatada

Representando relatos de humor depressivo, independente de estarem refletidos na aparência. Inclui abatimento, desânimo ou sentimento de desamparo e desesperança. Avalie pela intensidade, duração e grau com que se relata que o humor é influenciado pelos acontecimentos. | ☐ 0. Tristeza ocasional compatível com as circunstâncias.
☐ 1.
☐ 2. Triste e abatido, mas se alegra sem dificuldades.
☐ 3.
☐ 4. Sentimentos predominantes de tristeza ou melancolia. O humor é ainda influenciado por circunstâncias externas.
☐ 5.
☐ 6. Tristeza, infelicidade ou desânimo contínuos e invariáveis. |
| 5) | 3. Tensão Interior

Representando sentimentos de desconforto indefinido, inquietação, agitação interior, tensão mental crescente chegando até pânico, pavor ou angústia. Avaliar de acordo com intensidade, frequência, duração do grau de reassuramento necessário. | ☐ 0. Tranquilo, somente tensão interior fugaz.
☐ 1.
☐ 2. Sentimentos ocasionais de inquietação e desconforto indefinido.
☐ 3.
☐ 4. Sentimentos contínuos de tensão interna ou pânico intermitente que o paciente só consegue dominar com alguma dificuldade.
☐ 5.
☐ 6. Apreensão ou angústia persistente. Pânico incontrolável. |
| 6) | 4. Sono Diminuído

Representando a experiência de redução de duração ou profundidade do sono comparadas com o padrão normal próprio do indivíduo quando está bem. | ☐ 0. Dorme normalmente
☐ 1.
☐ 2. Leve dificuldade para adormecer ou sono discretamente reduzido, leve ou interrompido.
☐ 3.
☐ 4. Sono reduzido ou interrompido por, pelo menos, duas horas.
☐ 5.
☐ 6. Menos de duas ou três horas de sono. |

-
- 7) 5. Diminuição Do Apetite
- Representando o sentimento de perda de apetite quando comparado ao seu normal. Avalie pela perda da vontade de comer ou pela necessidade de forçar-se a comer.
- ☐ 0. Apetite normal ou aumentado.
 - ☐ 1.
 - ☐ 2. Apetite levemente diminuído.
 - ☐ 3.
 - ☐ 4. Sem apetite. A comida não tem sabor.
 - ☐ 5.
 - ☐ 6. É necessário ser sempre persuadido para comer.
-
- 8) 6. Dificuldades de Concentração
- Representando dificuldades em concluir ou organizar os pensamentos chegando à falta de concentração incapacitante. Avalie de acordo com a intensidade, a frequência e o grau de incapacidade resultante.
- ☐ 0. Sem dificuldade para se concentrar.
 - ☐ 1.
 - ☐ 2. Dificuldades ocasionais em concluir ou organizar os pensamentos.
 - ☐ 3.
 - ☐ 4. Dificuldades para se concentrar e sustentar o pensamento, que reduzem a capacidade para ler ou manter uma conversa.
 - ☐ 5.
 - ☐ 6. Incapaz de ler ou conversar, a não ser com grande dificuldade.
-
- 9) 7. Lassidão
- Representando a dificuldade ou a lentidão para iniciar e realizar atividades rotineiras.
- ☐ 0 Dificilmente apresenta qualquer dificuldade para iniciar atividades. Sem preguiça.
 - ☐ 1.
 - ☐ 2. Dificuldades para iniciar atividades.
 - ☐ 3.
 - ☐ 4. Dificuldades para começar atividades rotineiras simples, que são realizadas à custa de esforço.
 - ☐ 5.
 - ☐ 6. Lassidão completa. Incapaz de fazer qualquer coisa sem ajuda.
-
- 10) 8. Incapacidade de Sentir
- Representando a experiência subjetiva de interesse reduzido pelo ambiente ou por atividades que são normalmente prazerosas. A capacidade de reagir com emoção apropriada às circunstâncias ou às pessoas está reduzida.
- ☐ 0. Interesse normal pelo ambiente e pelas outras pessoas.
 - ☐ 1.
 - ☐ 2. Capacidade reduzida de desfrutar interesses rotineiros.
 - ☐ 3.
 - ☐ 4. Perda de interesse pelo ambiente. Perda de sentimentos pelos amigos e conhecidos.
 - ☐ 5.
 - ☐ 6. A experiência de estar emocionalmente paralisado, incapaz de sentir raiva, pesar ou prazer e uma falta de sentimentos completa ou mesmo dolorosa em relação a parentes próximos ou amigos.
-
- 11) 9. Pensamentos Pessimistas
- Representando pensamentos de culpa, inferioridade, auto.reprovação, pecado, remorso e ruína.
- ☐ 0. Sem pensamentos pessimistas.
 - ☐ 1.
 - ☐ 2. Ideias flutuantes de falha, auto-reprovação ou autodepreciação.
 - ☐ 3.
 - ☐ 4. Autoacusações persistentes ou ideias definidas, mas ainda racionais de culpa ou pecado. Progressivamente pessimista sobre o futuro.
 - ☐ 5.
 - ☐ 6. Delírios de ruína, remorso ou pecado irremediável. Autoacusações que são absurdas e inabaláveis.

12) 10. Pensamentos Suicidas

Representando o sentimento de que não vale a pena viver, que uma morte natural seria bem.vinda, pensamentos suicidas e preparativos para suicídio. Tentativas de suicídio por si só não devem influenciar a avaliação.

- ☐ 0. Aprecia a vida ou a aceita como ela é.
- ☐ 1.
- ☐ 2. Enfastiado de viver. Pensamentos suicidas transitórios
- ☐ 3.
- ☐ 4. Provavelmente seria melhor morrer. Pensamentos suicidas são frequentes e o suicídio é considerado com uma solução possível, mas sem planos ou intenções específicas.
- ☐ 5.
- ☐ 6. Planos explícitos para o suicídio quando houver uma oportunidade. Providência para o suicídio.

1 9.3.4. Udvalg for Kliniske Undersøgelser Side Effect Rating scale

Escala de efeitos colaterais (UKU-SERS-PAT)

Page 1

1) Nome completo

2) Data

VERIFIQUE A CAIXA APROPRIADA PARA CADA ITEM.

AS QUATRO ALTERNATIVAS DE RESPOSTA NA FORMA DO PACIENTE CORRESPONDEM A GRAUS DE GRAVIDADE (0-1-2-3).

Informações sobre a relação causal podem ser obtidas entrevistando o paciente após o preenchimento da escala de autoavaliação.

	Sintomas: 0	Sintomas: 1	Sintomas: 2	Sintomas: 3	Relação causal: Impossível	Relação causal: Possível	Relação causal: Provável
3) 1.1 Dificuldades de concentração	☐	☐	☐	☐	☐	☐	☐
4) 1.2 Astenia / Laxidão / Maior fadigabilidade	☐	☐	☐	☐	☐	☐	☐
5) 1.3 Sonolência / Sedação	☐	☐	☐	☐	☐	☐	☐
6) 1.4 Memória insuficiente	☐	☐	☐	☐	☐	☐	☐
7) 1.5 Depressão	☐	☐	☐	☐	☐	☐	☐
8) 1.6 Tensão / Inquietação interna	☐	☐	☐	☐	☐	☐	☐
9) 1.7 Maior duração do sono	☐	☐	☐	☐	☐	☐	☐
10) 1.8 Duração reduzida do sono	☐	☐	☐	☐	☐	☐	☐
11) 1.9 Aumento da atividade dos sonhos	☐	☐	☐	☐	☐	☐	☐
12) 1.10 Indiferença emocional	☐	☐	☐	☐	☐	☐	☐
13) 2.1 Distonia	☐	☐	☐	☐	☐	☐	☐
14) 2.2 Rigidez	☐	☐	☐	☐	☐	☐	☐
15) 2.3 Hipocinesia / Acinesia	☐	☐	☐	☐	☐	☐	☐
16) 2.4 Hipercinesia	☐	☐	☐	☐	☐	☐	☐
17) 2.5 Tremor	☐	☐	☐	☐	☐	☐	☐
18) 2.6 Acatisia	☐	☐	☐	☐	☐	☐	☐
19) 2.7 Ataques epilépticos	☐	☐	☐	☐	☐	☐	☐
20) 2.8 Parestesias	☐	☐	☐	☐	☐	☐	☐
21) 2.9 Dor de cabeça	☐	☐	☐	☐	☐	☐	☐
22) 3.1 Distúrbios na acomodação	☐	☐	☐	☐	☐	☐	☐
23) 3.2 Aumento da salivação	☐	☐	☐	☐	☐	☐	☐
24) 3.3 Salivação reduzida (boca seca)	☐	☐	☐	☐	☐	☐	☐
25)							

3.4 Náusea / vômito	☐	☐	☐	☐	☐	☐	☐
26) 3.5 Diarreia	☐	☐	☐	☐	☐	☐	☐
27) 3.6 Prisão de ventre	☐	☐	☐	☐	☐	☐	☐
28) 3.7 Perturbações de micção	☐	☐	☐	☐	☐	☐	☐
29) 3.8 Poliúria / Polidipsia	☐	☐	☐	☐	☐	☐	☐
30) 3.9 Tortura ortostática	☐	☐	☐	☐	☐	☐	☐
31) 3.10 Palpitações / taquicardia	☐	☐	☐	☐	☐	☐	☐
32) 3.11 Maior tendência para suar	☐	☐	☐	☐	☐	☐	☐
33) 4.1 Irritação na pele	☐	☐	☐	☐	☐	☐	☐
34) 4.2 Prurido	☐	☐	☐	☐	☐	☐	☐
35) 4.3 Fotossensibilidade	☐	☐	☐	☐	☐	☐	☐
36) 4.4 Pigmentação aumentada	☐	☐	☐	☐	☐	☐	☐
37) 4.5 Ganho de peso	☐	☐	☐	☐	☐	☐	☐
38) 4.6 Perda de peso	☐	☐	☐	☐	☐	☐	☐
39) 4.7a Menorragia	☐	☐	☐	☐	☐	☐	☐
40) 4.7b Metrorragia	☐	☐	☐	☐	☐	☐	☐
41) 4.8 Amenorreia	☐	☐	☐	☐	☐	☐	☐
42) 4.9 Galactorreia	☐	☐	☐	☐	☐	☐	☐
43) 4.10 Ginecomastia	☐	☐	☐	☐	☐	☐	☐
44) 4.11 Desejo sexual aumentado	☐	☐	☐	☐	☐	☐	☐
45) 4.12 Desejo sexual diminuído	☐	☐	☐	☐	☐	☐	☐
46) 4.13 Disfunção erétil	☐	☐	☐	☐	☐	☐	☐
47) 4.14a Disfunção ejaculatória	☐	☐	☐	☐	☐	☐	☐
48) 4.14b Ejaculação precoce	☐	☐	☐	☐	☐	☐	☐
49) 4.15 Disfunção orgásmica	☐	☐	☐	☐	☐	☐	☐
50) 4.16 Vagina seca	☐	☐	☐	☐	☐	☐	☐