HOW TO USE TRANSCRANIAL FOCUSED ULTRASOUND NEUROMODULATION TO 

ENHANCE MINDFULNESS 

by 

Brian Lord 

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Dedication

May this work be a humble offering to the divine spirit of God that inspires and animates All. May it serve the pursuit of Truth, Goodness, and Beauty. May it serve the true spirit of science.
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Abstract

This investigation explores the application of non-invasive brain stimulation (NIBS), specifically transcranial focused ultrasound (tFUS), to enhance mindfulness and meditation practices. The research investigates the potential of tFUS to modulate brain activity, particularly within the Default Mode Network (DMN) and the posterior cingulate cortex (PCC), areas linked to self-referential thoughts and the sense of self, which are often targeted in meditation practices.

The dissertation comprises three main studies. The first chapter is a scoping review that synthesizes current research on enhancing mindfulness through various NIBS techniques, focusing on their methodologies and effectiveness in doing so. The review concludes that while most studies target cognitive control enhancements via the dorsolateral prefrontal cortex (DLPFC), a more promising approach could be the suppression of PCC and DMN activity to aid in improving mindfulness skills.

The second and third chapters detail empirical studies where tFUS is applied directly. The second study, a pilot study using fMRI, assesses the effects of tFUS on the DMN in healthy subjects, demonstrating that tFUS targeted at the PCC can reduce DMN functional connectivity and enhance the subjective experience of mindfulness. The third study employs concurrent EEG and tFUS in experienced meditators to build on the previous findings, though it faces technical challenges that limit its conclusive power.

Overall, the dissertation aims to bridge gaps between neuromodulation technologies and mindfulness practices, suggesting that tFUS could be a powerful tool for enhancing mental training techniques inherent in meditation. The research underscores the potential for NIBS paradigms that manipulate subjective experiences to be used not only to improve mental health outcomes, but to advance the cognitive neuroscience of meditation and consciousness by contributing to the development of causal theoretical models. The findings invite further
investigation into the mechanisms of tFUS and its broader applications in scientific and therapeutic settings.
Introduction

Since the dawn of time, questions regarding the nature of mind, self, and consciousness have been central to human’s existence. They have been integral to the science of psychology since its materialization in the late 19th century, notably examined in William James’s textbook *The Principles of Psychology* (1890). His emphasis on the importance of introspection, attention, and the mysterious unity of consciousness explicitly draws attention to the boundaries of science and human knowledge itself, raising philosophical considerations that reference Descartes’ mind-body problem and Kant’s transcendental idealism (Goodman, 2021). These considerations are also present in the much more ancient lineage of contemplative traditions out of which the practice of meditation emerged in the East, particularly Buddhism (Grossman & Van Dam, 2011). In recent decades, the science of meditation and mindfulness has emerged as a dominant force in the field of psychology, renewing these ages-old questions in the context of modern neuroscience (Brandmeyer et al., 2019; Kabat-Zinn, 2019).

While much has been discovered about the physical structure and activity of the brain, the question of how those physical changes translate to subjective experience and vice-versa – the so-called hard problem of consciousness – remains an incommensurable challenge (Chalmers, 1995). Whether the activity that is responsible for the transduction between physical substance and subjective experience is the informational dynamics of neural firing (Doerig et al., 2021), the microtubule processing network within neural tissue (Hameroff & Penrose, 2014), a nebulous physical field that intersects with biological tissue (Jibu et al., 1996; Meijer et al., 2021), or something else entirely, it is not beyond the realm of science. There must be a solution that involves brain activity, given that experience can alter brain activity, and brain activity can alter experience. It is the latter scenario, in which altering brain activity alters experience, that is the domain of this investigation.
Non-invasive brain stimulation (NIBS) encompasses a family of neuromodulation techniques that can temporarily alter brain activity by applying elemental physical forces like magnetism (transcranial magnetic stimulation – TMS), electricity (transcranial electrical stimulation – TES), light (photobiomodulation – PBM), or sound (transcranial ultrasound – TUS) to the scalp (Polania et al., 2018). These techniques have proven therapeutic and neuroenhancement benefits (Antal et al., 2022; Lewis et al., 2016). Perhaps the most promising form of NIBS is transcranial focused ultrasound (tFUS), due to its ability to target subcortical sites with high spatial specificity (Blackmore et al., 2019). Its mechanisms are complicated: translating from acoustic energy to changes in neural firing does not follow a singular or obvious path. Clarifying these mechanisms is an important problem to solve to derive maximal benefits from the technology, and it is one of the motivators of this dissertation. In general, it is likely that the applications and benefits of NIBS will expand and improve as the techniques are refined, leading to progress in the future field of qualia engineering – orchestrated modulation of experience – as consciousness problems get more tractable.

William James emphasized the importance of introspection because it refines our ability to process our stream of consciousness, allowing us to better understand our mind. If he were alive today, he would likely be enthusiastic about the progress of contemplative neuroscience, which aims to unite the experiential knowledge of mindfulness with neuroscience’s understanding of the brain. Central to the practice of meditation is the idea of the illusion of the self, both in the context of narratives about “me” and in the experience of “I” in agency and sensation. Both contemplative traditions and contemporary neurocognitive science teach that the self and the world is a contextual construction, that its continuity and solidity is a mirage (Hoffman, 2019; Shamil Chandaria, 2024).
In the brain, the primary functional network underlying the experience of self is the default mode network (DMN), which is associated with mind-wandering, narrative formation, and memory integration (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014; Davey et al., 2016; Raichle, 2015). Not coincidentally, alterations in DMN are associated with meditative practice – it is hypothesized that the central executive network (CEN) acts to inhibit the DMN during meditation (Bauer et al., 2019; Panda et al., 2016). More specifically, the posterior cingulate cortex (PCC) has been associated with the self and “getting caught up in” experience, and meditation suppresses these functions (J. Brewer et al., 2013; J. A. Brewer et al., 2011; J. A. Brewer & Garrison, 2014). This is critical to the concept of mindfulness, which is generally understood to be the capacity to be deliberately present in the moment without judgment (Kabat-Zinn, 1995).

Given these insights, it is conceivable that NIBS could be used to enhance meditation and mindfulness. The first chapter of this dissertation is a scoping review examining empirical studies that do that. Nearly all of them take the approach of enhancing executive functioning. The review concludes by arguing that a more amenable approach would be to suppress PCC and DMN activity. Such an approach is taken in the following two chapters, in which empirical experiments were conducted on the effects of tFUS when applied to the PCC with the intent to suppress or disrupt activity there. The first experiment is an fMRI pilot study that tested whether such a protocol works, and in participants that received active stimulation, it found evidence of reductions in DMN functional connectivity paired with increases in mindfulness and alterations in sense of ego and time. The second experiment utilized concurrent EEG paired with PCC-targeted tFUS to build on the lessons previously learned. This study faced considerable technical challenges and found minimal effects.
Overall, this investigation is intended to advance our understanding of the mind and consciousness by exploring the use of NIBS (specifically tFUS) to alter functioning at the highest level of the neurocognitive hierarchy (the DMN) to influence some of the deepest aspects of our experience. The principal focus throughout this entire investigation was the first word of the title of dissertation: How. It was intended to be a methodological and mechanistic research project with a pragmatic approach: *How* does tFUS alter brain activity? *How* can we measure its effects? *How* can we measure aspects of subjective experience or mindfulness skills? *How* does mindfulness even work? *How* do we become better at it? Can we put all these questions together, and ask: *How* can we use NIBS to enhance mindfulness? This is the framework from which decisions were made throughout this research.
Chapter 1: Enhancing Mindfulness with Non-Invasive Brain Stimulation: A Scoping Review

This paper (see appendix A) was submitted as an invited review to a special issue on mindfulness in the journal Biological Psychiatry. It serves as a literature review and theoretical justification for the two empirical studies that follow. Given that NIBS has shown potential in boosting neurocognitive functions and addressing clinical symptoms, this scoping review was designed to investigate how these techniques can accelerate the acquisition of mindfulness skills.

The review surveys available literature to find all empirical studies that use NIBS to measure changes in mindfulness, analyzing the effectiveness and methodologies employed. Nearly all the studies targeted the dorsolateral prefrontal cortex (dlPFC) to enhance executive functioning. Only TMS and TES were used; no studies used tFUS. While some studies indicated positive outcomes, the overall field lacks consistency and depth in results due to variable experimental designs, imprecise psychometric measurements, and differing stimulation parameters. The review suggests that future research should aim for more sophisticated NIBS applications and a finer-grained approach to defining and measuring mindfulness, proposing a shift towards focusing on the skills underpinning mindfulness such as attention control and self-regulation.

The review emphasizes the potential of NIBS as a valuable tool for enhancing mindfulness training but calls for more rigorous approaches to establish reliable protocols. It concludes by arguing that, along with improvements in NIBS techniques, theoretical conceptions of mindfulness must be improved. Namely, there is the problem that mindfulness operates as an underspecified term. Pointing to more specific focus skills like concentration, clarity, and equanimity highlights the actual neurocognitive processes underlying meditation and mindfulness. This then lays the groundwork for clearly defined theoretical motivations for targeting specific brain sites with NIBS. The review closes by suggesting that suppressing the
PCC to enhance equanimity is a more promising approach to mindfulness enhancement than stimulating dlPFC.

In terms of my contributions to this study, I designed the protocol for the scoping review. Co-authors Sanguinetti and Young informed me that they were invited to write a review almost identical to my proposal, so we agreed to join forces on the matter. I led the writing, literature review, and analysis, with their advice. Their theoretical ideas took prominence in the discussion section, in which it is declared that mindfulness critically must be understood as a trainable set of skills, and that equanimity is perhaps the most important of these skills. Co-author Allen provided regular oversight and advice as the paper was formulated, developed, and written.
Chapter 2: Transcranial Focused Ultrasound to the Posterior Cingulate Cortex Modulates Default Mode Network: A Pilot Study

This paper (see appendix B) was submitted to a special issue on focused ultrasound in the journal Frontiers in Neurosciences. It investigates the effects of tFUS when targeting the PCC. It was designed as a pilot study to test the feasibility of the proposed design. The experiment used a randomized single-blind design with thirty healthy subjects, with participants receiving either active or sham tFUS. Resting-state BOLD signal fMRI was collected before and after the administration of tFUS to detect changes in functional connectivity as a result of the sonication. It also recorded the subjective effects of tFUS on mindfulness, mood, and self-related processing using several psychometric scales. It was hypothesized that the tFUS would temporarily reduce functional connectivity in the DMN and enhance state-level mindfulness and mood.

Results revealed that the active tFUS group exhibited significant reductions in midline DMN functional connectivity, suggesting effective neuromodulation of this network. In terms of subjective effects, individuals in the active group reported temporary increases in mindfulness and changes in self-awareness, including altered sense of ego and time, which were not observed in the sham group. These findings provided support for the core of the main hypothesis, that tFUS could be used to suppress the PCC and DMN to enhance mindfulness.

The implications of these findings are profound, indicating that tFUS could serve as a non-invasive method to potentially treat psychiatric and neurological conditions associated with DMN dysfunctions, such as depression and anxiety. Given that it was a simple pilot study, the discussion section underscores the need for further research to replicate these results in larger samples and to refine the tFUS protocol for more consistent effects. Additionally, it suggests exploring the therapeutic benefits of neuromodulation techniques in clinical settings, given the ability of tFUS to target deep brain structures selectively and non-invasively. Finally, seeing
these results in a pre-post design raises the question of what is happening during stimulation, which is addressed in the next experiment.

In terms of my contribution to this study, co-authors Sanguinetti, Young, and Allen designed the protocol. I helped run it with co-authors Sanguinetti and Ruiz, learning how to administer tFUS during this study. I spearheaded analysis and led authorship of the paper, under the tutelage of co-authors Sanguinetti and Allen.
Chapter 3: Psychophysiological Effects of Transcranial Focused Ultrasound on Experienced Meditators

This paper (see appendix C) was prepared for submission to PsyArXiv. Building on the previous work, it investigates the effects of tFUS on meditation by applying it to experienced meditators. Whereas the previous study used meditation-naïve subjects and still detected a mindfulness increase, this study was designed around meditation practice. Fifteen experienced meditators were recruited to participate in three sessions each, composed of a sham condition and two different pulse repetition frequencies (PRFs). Participants performed a body scan meditation while EEG and EKG data were collected before, during, and after tFUS, allowing for a concurrent EEG-tFUS design. Subjective measures were also collected after the meditation. The aim was to replicate and build upon previous results showing mindfulness enhancement, to test the effects of differing PRFs, and to investigate the online effects of tFUS on EEG signals.

Focus was put on potential mechanisms of tFUS. It is a difficult task to integrate the occasionally clashing and incongruous results across the literature. The picture changes at the cellular level, tissue level, and whole brain level, due to the battery of mechanisms. Working from the level of EEG, it was hypothesized that the suppressive tFUS might act by disrupting phase synchronization, thereby disrupting the ability for distant brain regions to communicate.

Results from the study found minimal changes across most measurements. No significant differences were observed in frontal alpha power, frontal theta power, HRV metrics, EEG phase rate, and EEG complexity metrics. However, one finding was a reduction in inter-site phase clustering (ISPC) in the alpha frequency range in the high pulse PRF condition, suggesting a clue into the underlying mechanisms of tFUS that warrants further research.

The findings are compromised by several major limitations, including issues with the tFUS equipment, discomfort reported by participants that may have prevented their ability to
meditate well, and the fact that individual structural MRIs were not used for precise targeting. The paper contributes to the understanding of neuromodulation's role in enhancing meditation, proposing that while tFUS presents a promising tool for influencing mental states, sensible experimental design and careful maintenance of delicate technology are required to harness its full potential. This study lays the groundwork for future research into the intersections of neuromodulation, meditation, and consciousness, with a particular focus on the methodological rigor needed to clarify these complex interactions.

In terms of my contributions to this study, I helped design it with co-authors Sanguinetti, Fini, and Allen. I helped run it with co-authors Fini, Beaman, and Cook. I led all analysis and writing, under the tutelage of co-authors Sanguinetti and Allen.
Conclusion

In summary, a scoping review was conducted on the potential to use NIBS to enhance mindfulness, with the literature showing mixed results. It was suggested that tFUS was an ideal candidate for this job due to its ability to target subcortical structures like the PCC, which is central to the behavior of “getting caught up in” experience, something that mindfulness practice trains you out of. Therefore, it was proposed that suppression of the PCC and DMN with tFUS may alter neurological activity in a way that enhances equanimity and mindfulness. An fMRI pilot study was performed to test this hypothesis, and it found support in both the neuroimaging data and the subjective measures. An EEG study was then performed to expand upon these promising results, and it faced numerous limitations and technical challenges that prevented any meaningful effects from being detected.

Overall, this work was intended to advance our understanding of the mind and consciousness, and it has served its purpose in that regard. Science developed with NIBS techniques promises real causal models of the relationship between functional brain activity and subjective experience. This type of science combined with the science of meditation, which dutifully probes the nature of not just the experience but also the experiencer, represents the forefront of psychological science. However, there are several methodological improvements that could be made on our approach.

Future Considerations in tFUS Methodology

First, the individualized targeting of the beam can be improved. In the MRI study, we used individual anatomic imaging to locate the ventral PCC in the neuronavigation software, but no modeling was performed to minimize skull aberration. Because individual skull shapes vary so widely in thickness, shape, and size, any given skull will defocus and deflect the ultrasound beam in a unique way. This must be accounted for to improve targeting accuracy across
individual participants. MRI imaging techniques like UTE (ultrashort echo-time) MRI and PETRA (Pointwise Encoding Time reduction with Radial Acquisition) MRI allow for accurate imaging of the cranium, which can then be used to model beam propagation inside the skull (Angla et al., 2023; Grodzki et al., 2012; Miller et al., 2015). Many methods have been developed to model and correct for skull aberration, including ray tracing (Jin et al., 2020), finite difference time domain (FDTD) (Connor et al., 2002), pseudo-spectral time domain (PSTD) (Robertson et al., 2017), and hybrid angular spectrum (HAS) (Leung et al., 2019).

These improvements could be taken further when paired with a functional-based targeting approach. DMNs identified in individuals using single-subject independent component analysis (ICA) show unique variations that are consistent in the same person across multiple scans, suggesting stable, individual differences in resting state functional networks (Meindl et al., 2010). The target for tFUS disruption could be selected based on peak activation in DMN or PCC using this approach. Targeting the peak of activation in the DMN may further enhance the effectiveness of the tFUS intervention by disrupting the region with the highest level of interconnectedness and synchrony.

Another way to further individualize the tFUS intervention may involve tuning the parameters of the ultrasound signal to intrinsic properties of the individual’s neural oscillations. Sanguinetti (2022) suggested that the PRF could be tuned to the individual alpha frequency (IAF), akin to a similar paradigm in rhythmic TMS (Thut et al., 2011). We have performed an experiment that tested this idea, targeting the PCC, which is an alpha burst generator (Rusiniak et al., 2018), and we detected significant increases in alpha frequency but not power (Lord et al., in prep). It is unclear how such modulations of neural oscillations might translate to behavior or subjective experience, but IAF, which is a highly heritable stable trait (Smit et al., 2006), is
associated with general cognitive ability and visual processing precision (Grandy et al., 2013; Haegens et al., 2014; Tarasi & Romei, 2024).

**Future Considerations in Mindfulness Research**

There are two major components to the mindfulness aspect of this research that can be improved: measurement and training. Measurement of mindfulness presents a difficult challenge (Baer, 2011; Hill & Labbé, 2014). Beginning with the fact that rigorously defining mindfulness reveals that it contains a battery of underlying components that are more aptly understood as skills (e.g., concentration, clarity, and equanimity), the experience of mindful states is likely driven by the engagement of these underlying skills (Young, 2016). Asking individuals to reflect on their subjective experiences through a survey instrument is not the same thing as measuring their abilities at a skill. This reveals a more precise question – is tFUS enhancing the subjective experience of mindful states or the underlying skills of mindfulness?

Techniques like experience tracing may present a finer-tuned methodology for tracking the subjective experience of meditation, tracking qualities like clarity, depth, wakefulness, boredom, or dereification (Cain et al., 2024; Jachs et al., 2022). It also allows for estimation of time spent “on-task” or “off-task” when the practitioner is tasked with meditating. Another approach is to use an in-depth questionnaire like the Lyon Assessment of Meditation Phenomenology (LAMP), which assesses a battery of phenomenological domains, including attentional, affective, and meta-cognitive (Abdoun et al., 2024). These approaches allow for a more straightforward assessment of the experience of the practitioner, sidestepping concerns about whether such effects pertain to a ‘mindfulness’ construct.

As it pertains to the skilled aspect of mindfulness, behavioral tasks that assess such skills may be of use. The salivary cortisol response to a stress test like the Trier Social Stress Test (TSST) may be an indicator of equanimity, and research shows that meditation interventions
improve stress-buffering responses to the induced stress (Lindsay et al., 2018; Morton et al., 2020). Tasks that test emotional or attentional regulation may also reflect mindfulness skills (Menezes et al., 2013). A neural stability signature may be another marker of the development of these skills (Bailey et al., 2024). Psychophysiological markers like electrodermal response, skin temperature, or event-related potentials could also be used to make inferences about equanimity (Delgado-Pastor et al., 2013; Manocha et al., 2010; Telles et al., 2013).

Finally, there exists a wide variety of meditation techniques and training styles, with varied goals, philosophies, and approaches to practice, and the field of contemplative science is still endeavoring towards a unified approach that can accommodate and organize this diversity (Matko & Sedlmeier, 2019; Nash et al., 2013; Reddy & Roy, 2019; Williams & Kabat-Zinn, 2011; Wright et al., 2023). This issue is not new and has been recognized since meditation became an object of scientific study (Bishop et al., 2004). However, in the specific context of training and purported enhancement of skills, it is likely that individual differences in intrinsic characteristics like personality and cognitive style play a mediating role in the effectiveness of any given intervention (de Vibe et al., 2015; Matko et al., 2022; Nyklíček & Irrmischer, 2017; van den Hurk et al., 2011). Therefore, the PCC-targeted tFUS intervention explored here may be appropriate for only certain individuals. Others may benefit more from stimulation to a different location. Similarly, different individuals may perform better or have improved subjective experiences according to the style of meditation performed. All these aspects of the varieties of mindfulness practice should be taken into consideration in the experimental design of future studies.
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Appendix A: Enhancing Mindfulness with Non-Invasive Brain Stimulation: A Scoping Review

Paper 1

Enhancing Mindfulness with Non-Invasive Brain Stimulation: A Scoping Review

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Abstract

Given the rise in popularity and benefits of mindfulness practices in various domains, including mental health, well-being, cognitive functioning, and physical health, any method that can accelerate or enhance mindfulness acquisition has wide-reaching implications. Non-invasive brain stimulation (NIBS) techniques, including transcranial magnetic stimulation (TMS), transcranial electrical stimulation (TES), and transcranial ultrasound stimulation (TUS), have shown promise in neurocognitive enhancement and clinical applications. This review aims to consolidate existing research on the intersection of NIBS and mindfulness, assessing the methodology, effectiveness, and commonalities of studies in this emerging field. A comprehensive literature search yielded 14 studies that met inclusion criteria, showing varied participant populations, methodologies, targets, and outcomes. Most studies focused on enhancing cognitive control processes through DLPFC stimulation, with varying levels of effectiveness. Predictably, interventions that were more intensive tended to be more effective, although there were some exceptions. The review highlights the embryonic state of this research area, emphasizing the necessity for more sophisticated NIBS techniques and a finer-grained approach to conceptualizing and assessing mindfulness. It suggests a potential shift towards targeting the focus skills underlying mindfulness rather than treating mindfulness only as a psychological state or trait that is experienced. Given that mindfulness is a component of several approaches to treating psychiatric disorders, this review and future research based on this review have translational implications for clinical practice.
Introduction

Over the first quarter of this century, meditation has exploded in popularity as a practical method for enhancing health and wellbeing (Kabat-Zinn, 2019b). While other more somatic practices like yoga and qi gong can be considered part of the larger family of meditative practices, mindfulness meditation has received the most attention in the psychological sciences. Mindfulness-Based Stress Reduction (MBSR) and Mindfulness-Based Cognitive Therapy (MBCT) are both standardized mindfulness training programs that have been used as interventions for psychiatric disorders with evidence of wide-ranging success (Alsubaie et al., 2017; Chiesa & Serretti, 2010). Broadly, mindfulness meditation practices are currently understood as mental training regimes aimed at improving awareness and self-regulation through attentional control, clarified awareness, and equanimity (Bishop et al., 2004; Brandmeyer et al., 2019; Giraldi, 2019; Lutz et al., 2015; Tang et al., 2015; Young, 2016).

Mindfulness, then, can be understood as the trainable capacity for being deliberately, fully present in the moment without judgment (Kabat-Zinn, 1995; Young, 2016). A quality that can be both state and trait, it has been operationalized for measurement with many instruments assessing diverse factors, including metacognition (Solem et al., 2015), attentional awareness (Black et al., 2012), embodiment (Khoury, 2018), curiosity (M. A. Lau et al., 2006), and emotional regulation (Feldman et al., 2007). Understandably, modern psychology has struggled to clearly and rigorously define it, given the elusive meaning of mindfulness even in the Buddhist traditions from which it has been derived. Much like beginners who are unsure of what to do when they first sit on a meditation cushion, psychological science has had learn as it goes how to develop methods to measure mindfulness accurately. Despite the limitations of self-report and psychometric instruments, they achieve pragmatic insights suitable for an emerging
discipline (Ackerman, 2017; Andrei et al., 2016; Baer, 2011; Grossman, 2008; Williams & Kabat-Zinn, 2011).

Mindfulness practices have been found to improve outcomes in stress and well-being (Goyal et al., 2014), emotionality, relationship issues, and attention (Sedlmeier et al., 2012), inflammation, immune response, and aging (Black & Slavich, 2016), psychopathology (Wielgosz et al., 2019), spiritual development and expanded consciousness (Vieten et al., 2018), and even epigenetic expression (Venditti et al., 2020). Given these wide-ranging benefits, mindfulness practices have been adopted in such diverse arenas as education (N. Lau, 2009), healthcare (Raski, 2015), correctional facilities (Samuelson et al., 2007), workplaces (Good et al., 2016), sports performance (Bühlmayer et al., 2017), creative arts (Schaewe, 2014), and sustainability (Wamsler et al., 2017). Any intervention that can accelerate or enhance the acquisition of mindfulness skills would have far-reaching potential across these domains and more.

Non-invasive brain stimulation (NIBS), which includes transcranial magnetic stimulation (TMS), transcranial electrical stimulation (TES), and transcranial ultrasound stimulation (TUS), has also emerged as a family of neuromodulation techniques with clinical and neurocognitive applications (Lewis et al., 2016; Polania et al., 2018; Rossini et al., 2015; Sanches et al., 2021). It has been shown to improve cognitive functioning in many brain disorders (Begemann et al., 2020), memory in aging (Goldthorpe et al., 2020), motor functioning in stroke and Parkinson’s (Schulz et al., 2013), and can function as a treatment for depression, anxiety, and neuropathic pain (Rossini et al., 2015; Vicario et al., 2019).

Some of the biggest promises from NIBS involve the idea of neuroenhancement of higher cognitive processes such as working memory and executive functioning (Antal et al., 2022;
Brunoni & Vanderhasselt, 2014; de Boer et al., 2021). While some studies and meta-analyses do show positive results in these domains, the wide parameter space, expectation effects, task differences, and individual differences make it difficult both to develop a protocol that consistently works and to evaluate the limitations of any paradigm (Antal et al., 2022; Braga et al., 2021; Sanches et al., 2021). Nevertheless, the field is evolving its methodologies to address these challenges (Fins et al., 2017).

Given these successes, there is the possibility that NIBS can be used to enhance mindfulness training and interventions. A recent review evaluated the outcome effects of the combination of TES and meditation (Divarco et al., 2023), and an upcoming review evaluates the mental health outcome effects of NIBS combined with meditation practice (Hunter, 2016). The question remains whether NIBS can be used to change state levels of mindfulness or a participant’s baseline level of trait mindfulness over time, whether synergistically in the context of training or independently of it. This scoping review aims to organize all such studies that have measured mindfulness changes as a result of NIBS and evaluate their commonalities and differences in methodology and effectiveness. This will serve as a foundation for future development of NIBS-based mindfulness enhancement paradigms, which have a range of applications from promoting wellbeing to alleviating psychiatric symptoms.

Methods

Literature searches were conducted in February 2024. PubMed and PsycINFO were used. These databases were chosen for their comprehensive coverage of medical, health sciences, and psychological literature, respectively. The same keywords were used for both databases.

The following search string was used: ((("transcranial electrical stimulation") OR ("transcranial direct current stimulation") OR (TDCS) OR ("transcranial alternating current stimulation") OR (TACS) OR (“transcranial random noise stimulation”) OR (TRNS) OR ("non-
invasive brain stimulation") OR ("brain stimulation") OR ("neuromodulation") OR ("transcranial
magnetic stimulation") OR (TMS) OR ("theta-burst stimulation") OR (TBS) OR (RTMS) OR
(photobiomodulation) OR (PBM) OR ("transcranial infrared") OR (TILS) OR ("transcranial
laser") OR ("low-level laser therapy") OR (LLLT) OR ("transcranial ultrasound") OR
("transcranial focused ultrasound") OR (TFUS) OR (TUS) OR (LIFUS) OR ("low intensity
focused ultrasound") OR ("focused ultrasound neuromodulation") OR (FUN) OR ("focused
ultrasound") AND ((mindfulness) OR (mindful) OR (meditation))

The search and screening process is represented in a PRISMA flow diagram (see figure
1). Studies were evaluated based on the following inclusion criteria: 1) empirical study on human
subjects, 2) some form of NIBS (TES, TMS, TUS, or PBM) was used, 3) some form of
measurement of mindfulness as an outcome. Both clinical and non-clinical studies and
populations were included. Studies were excluded if they: 1) used non NIBS methods for
alteration, such as neurofeedback, stimulation that does not target the brain (e.g., transcutaneous
electrical stimulation), purely sensory interventions (e.g., binaural beats, forest bathing, guided
meditations), acupuncture, or physical or behavioral therapy, 2) are non-empirical work such as a
review, or 3) inaccessible study materials.

Once eligible studies were identified, information about the design, participants,
intervention, mindfulness outcomes, and other outcomes was extracted to be presented in table 1.
Effect sizes, when not specified, were calculated from available data using an online calculator
(Lenhard & Lenhard, 2022). A simplified table was also constructed (see table 2). This presents
the same information in an abridged fashion, denoting the number of participants, whether it was
a clinical study, the type of NIBS, the anatomical target of the NIBS, whether they meditated
during the study, and a rating on the successfulness of the intervention at enhancing mindfulness.
Results

Study Characteristics

Out of 422 records identified (see figure 1), 14 studies met all the inclusion criteria. These studies showed high variability across almost every metric of comparison. The number of subjects ranged from 3 to 89, with a total 449 subjects across all studies, and a mean value of 32 subjects per study. Ten studies used tDCS as their NIBS intervention, and four studies used rTMS. No studies used TUS, PBM, or some other variant of TES or TMS. Eleven studies targeted some form of the DLPFC, two studies targeted the R-IFG, and one study targeted the M1 motor cortex. Ten studies were sham-controlled (nine of which were double-blinded and randomized, while one was single-blind and pseudo-random), two were open-label, and two were retrospective reviews of an intervention. Four were registered clinical trials. Nine studies had participants meditate during stimulation, three did not specify whether meditation was used, one employed hatha yoga, and one did not use meditation. Eight studies used mindfulness meditation, one used treadmill walking meditation, and one used a choice of either focused attention (FA) or open monitoring (OM) meditation. See table 1 for full summary of study characteristics and table 2 for a simplified summary.

This section will review these studies across three major themes: 1) their stimulation approach – what brain region did they target and how? 2) their measurement approach – how did they measure mindfulness? 3) their overall intervention approach – how many sessions were conducted? What kind of meditation instruction did participants receive?

Figure 1.
PRISMA Flow Diagram

Identification of studies via databases and registers

Identification

Records identified from*: PubMed (n = 283) PsychINFO (n = 139) Total (n = 422) → Records removed before screening: Duplicates (n = 46)

Screening

Records screened by title/abstract: (n = 376) → Reports excluded:
Not empirical study (n = 171)
No brain stimulation (n = 120)
No mindfulness outcome (n = 54)
No human model (n = 5)
Total (n = 350)

Reports screened by full text: (n = 26) → Reports excluded:
No brain stimulation (n = 2)
No mindfulness outcome (n = 10)
Total (n = 12)

Included

Studies included in review: (n = 14)

Note. PRISMA flow diagram of literature search process (Page et al., 2021)

Table 1: Summary of studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Design</th>
<th>Participants</th>
<th>Intervention</th>
<th>Mindfulness Outcomes</th>
<th>Other Outcomes</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Study</td>
<td>Underpinning the neurological source of executive function following cross-hemispheric tDCS stimulation.</td>
<td>Sham-controlled, single blind, pseudo-random assignment, pre-post measurements.</td>
<td>N=40, 22♂18♀, age=22±1.5. 19 active, 21 sham. Healthy, (min 20, max 30 years)</td>
<td>tDCS: target = bilateral DLPFC anode = F3, cathode = F4; length = 30 min. active condition = 2 mA sham condition = 2 mA for 30 s 5 consecutive days x 30 min sessions Unspecified what participants did during stimulation period</td>
<td>MAAS: Increase in active group, with significant interaction effect (d=0.55, p=0.05). Increases in theta, alpha, beta power in DLPFC, cingulate, and parietal cortex. Interaction effect showing increase in executive functioning in active group (Stroop task and 3-back)</td>
<td>No discernable difference between 1 mA and 2 mA in most conditions except 1 mA reduced sadness more than 2 mA</td>
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<tr>
<td>Study</td>
<td>A Double-Blind Study Exploring the Use of Transcranial Direct Current Stimulation (tDCS) to Potentially Enhance Mindfulness Meditation (E-Meditation).</td>
<td>Sham-controlled, double blind, cross over, randomized, registered clinical trial NCT02790619.</td>
<td>N=15, 8♂7♀, age=28.2±6.8. All received each condition. Healthy, no prior meditation experience</td>
<td>tDCS: target = R-DLPFC anode = F8, cathode = supraorbital region; length = 20 min. 3 conditions: 1 mA, 2 mA, sham 3 visits, 1-week apart, each condition once. Guided audio mindfulness meditation during stimulation</td>
<td>Toronto Mindfulness Scale: unchanged; FFMQ: significant increase in Acting with Awareness (d=2.6, p=0.0175) comparing sham to 1 mA, trend increase in Observing; VAS: trend decrease in sadness, excitedness, restlessness, trend increase in calmness</td>
<td>No discernable difference between 1 mA and 2 mA and 2 mA in most conditions except 1 mA reduced sadness more than 2 mA</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcomes</td>
<td></td>
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<tr>
<td>Brooks et al., 2021</td>
<td>Sham-controlled, double-blind, randomized, two-site, pilot. Registered clinical trial NCT03653351 and NCT03680664. Primarily a feasibility study for tDCS+MBSR</td>
<td>N=26, 14♂</td>
<td>12♀, age&gt;60. 12 active, 14 sham. Symptoms of depression or anxiety + subjective cognitive complaints but still intact cognitive functioning. No prior meditation experience.</td>
<td>tDCS: target = bilateral DLPFC anode = Fz, cathode = Iz; length = 30 min. active = 2 mA for 30 min, sham = 2 mA for 54 s 10-week MBSR course + daily 30-min tDCS during practice.</td>
<td>CAMS-R: Increase in both groups, but greater increase in active group Interaction effect (d=0.6, p=0.07). No statistically significant full effects, but moderate trends of reduction in anxiety, improvement in social functioning, and small trends reduction in depression (PROMIS) and improvement in cognitive performance (Fluid Cognition Composite)</td>
<td></td>
</tr>
<tr>
<td>Cavallero et al., 2021</td>
<td>Open-label, two-site, pilot, feasibility, clinical trial for treatment of MDD</td>
<td>N=27, 8♂</td>
<td>19♀, age=48.4±15.2. All active. Nonpsychotic, treatment-resistant MDD diagnosis.</td>
<td>rTMS: target=L-DLPFC 10-Hz at 120% intensity of MT for at least 3000 pulses, or 1-Hz to R-DLPFC at 120% intensity for 360 pulses, then 20-Hz to L-DLPFC at 120% intensity for 1200 pulses. 5-week MBCT audio-guided protocol during daily TMS sessions.</td>
<td>FFMQ: Significant increases in all 5 subscales Observing (d=0.4, p=0.02) Describing (d=0.41, p=0.01) Awareness (d=0.55, p=0.03) Nonjudgment (d=0.89, p&lt;0.0001) Nonreactivity (d=0.81, p=0.03) Significant reductions in depression severity (IDS-SR, PHQ-9) and perceived stress (PSS). Significant increases in quality of life (Q-LES-Q-SF), and 3 subscales of MAIA - attention regulation, self-regulation, and body listening.</td>
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</tr>
</tbody>
</table>

Variable adherence. Adverse effects were mild and transient. No full statistical significance, but trends suggest synergistic effect. 10 subjects withdrew from study, due to TMS discomfort, difficulty meditating, negative mood states. Dropouts showed no significant changes in clinical or mindfulness measures.
<p>| Danilewitz et al., 2021 | Effect of combined yoga and transcranial direct current stimulation intervention on working memory and mindfulness. | Sham-controlled, double-blind, randomized, cross-over to improve working memory and mindfulness. N=22, 14♂|8♀, age=29.1±4.6. All received each condition. Healthy, 2+ years of regular yoga practice, no prior meditation experience. | tDCS: target = bilateral DLPFC anode = F3, cathode = F4; length = 20 min. Active = 2 mA for 20 min with 30 s ramp up and down. Sham = 2 mA for 60s of ramp up and down. 2 visits at least a week apart. Unspecified what participants did during stimulation period. 40-min Hatha yoga session followed tDCS treatment. | Toronto Mindfulness Scale: Increase in both subscales for both active and sham groups. No interaction effect (d=0.132). No active vs sham effects on working memory (N-back). | 4 total dropouts. tDCS and yoga were not simultaneous. Yoga followed tDCS. |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Overview</th>
<th>Sample</th>
<th>Interventions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibson et al., 2022</td>
<td>Transcranial Direct Current Stimulation Provides no Additional Benefit to Improvements in Self-Reported Craving Following Mindfulness-Based Relapse Prevention.</td>
<td>Sham-controlled, double-blind, randomized trial to reduce alcohol craving and use</td>
<td>N=84, 50♂34♀. 47 active, 37 sham. Adults wanting to reduce their drinking</td>
<td>tDCS, target=r-IFG anode=r-IFG, cathode=left upper arm. Active = 2 mA for 30 min with 30 s ramp up and ramp down. Sham = 30 s ramp up and ramp down at first and last minute of 30 min period. MBRP guided meditation during stimulation. 8 weekly treatment sessions. Toronto Mindfulness Scale: No difference between active vs sham conditions in post-treatment (d=-0.1) High treatment adherence + sham tDCS significantly reduced post-treatment alcohol craving (PACS), while active tDCS showed no such effect</td>
</tr>
<tr>
<td>Hunter, 2016</td>
<td>Expanding your cognitive capacity: An assessment of the neuroplastic changes associated with mindfulness training and tDCS+MBT</td>
<td>Sham-controlled, double-blind, randomized to test cognitive enhancement with tDCS+MBT</td>
<td>N=29, 11♂18♀, age=27.6±5.6. 16 active, 13 sham. Healthy adults with no meditation training</td>
<td>tDCS, target=r-IFG, anode=F10, cathode=left upper arm; length = 30 min. Active = 2 mA for 30 min, Sham = 0.1 mA for 30 min. Guided meditation (either FA or OM) during. MAAS: No changes pre-post, no difference between conditions Enhancement in working memory (N-back and S-span), decoupling of DMN from focus-related processing</td>
</tr>
</tbody>
</table>

Dissertation. Mindfulness part of a larger study on high attrition rate (only 50 of 84 completed). Adherence had no effects on mindfulness.
<table>
<thead>
<tr>
<th>Study</th>
<th>Changes in mindfulness following repetitive transcranial magnetic stimulation for mood disorders.</th>
<th>N=32, 15♂17♀, age=50.0±13.8. All active. Patients with MDE diagnosis</th>
<th>rTMS, target=L-DLPFC. 10 Hz at 100% MT intensity for 3000 pulses, OR 1-Hz to R-DLPFC at 120% MT intensity.</th>
<th>Variable number of sessions, mean=33±7</th>
<th>FFMQ: significant increase in nonreactivity to inner experience subscale (d=0.976, p&lt;0.05)</th>
<th>Improvements in depression and anxiety (HRSD, PHQ-9, and GAD-7)</th>
<th>No meditation training. Subgroup analysis suggested improvements in decentering were independent of changes in depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leong et al., 2013</td>
<td>Retrospective chart review of outpatients treated for depression with rTMS</td>
<td>N=32, 15♂17♀, age=50.0±13.8. All active. Patients with MDE diagnosis</td>
<td>rTMS, target=L-DLPFC. 10 Hz at 100% MT intensity for 3000 pulses, OR 1-Hz to R-DLPFC at 120% MT intensity.</td>
<td>Variable number of sessions, mean=33±7</td>
<td>FFMQ: significant increase in nonreactivity to inner experience subscale (d=0.976, p&lt;0.05)</td>
<td>Improvements in depression and anxiety (HRSD, PHQ-9, and GAD-7)</td>
<td>No meditation training. Subgroup analysis suggested improvements in decentering were independent of changes in depression</td>
</tr>
<tr>
<td>McCallion et al., 2020</td>
<td>Efficacy of Transcranial Direct Current Stimulation-Enhanced Mindfulness-Based Program for Chronic Pain: a Single-Blind Randomized Sham Controlled Feasibility Trial</td>
<td>N=47, 15♂32♀, age=37.15±9.96. 24 active, 23 sham. Self-reported diagnosis of chronic pain</td>
<td>tDCS, target=dLPFC, electrodes on scalp and upper arm.</td>
<td>Active = 2 mA for 30 min, sham = 0.1 mA. MBP guided breath-focusing meditation during stimulation. 8 sessions over 4 weeks, with variable attendance.</td>
<td>MAAS: Significant interaction between groups attended and condition – those receiving active who attended more groups had higher scores (d=0.49, p=0.004)</td>
<td>Better health functioning (EQ-5D) also found with active condition and high attendance</td>
<td>23.4% dropout rate, mostly due to discomfort</td>
</tr>
<tr>
<td></td>
<td>Sham-controlled, double-blind, randomized feasibility trial to enhance mindfulness skills to help with chronic pain</td>
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<tr>
<td>Study</td>
<td>Design</td>
<td>Population</td>
<td>Intervention</td>
<td>Outcome Measures</td>
<td>Key Findings</td>
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<tr>
<td>Nishida et al., 2021</td>
<td>Sham-controlled, double-blind, randomized, to augment mindfulness effects and reduce anxiety</td>
<td>N=58, (age range 20-60). 28 active, 30 sham. Healthy adults</td>
<td>tDCS, target=L-DLPFC, anode=F5, cathode=left shoulder. Active = 1 mA for 20 min with 30s ramp up and ramp down. Sham = 1 mA for 30s in between the ramp up and ramp down. Guided treadmill walking focused meditation during stimulation.</td>
<td>FFMQ: no significant differences between active and sham</td>
<td>Roughly half of participants had moderate or high subclinical anxiety</td>
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<tr>
<td>Pimenta et al., 2021</td>
<td>Sham-controlled, double-blind, randomized clinical trial (NCT04219345) to reduce chronic migraine pain with mindfulness</td>
<td>N=30, all ♂. 16 active, 14 sham. 1+ year of diagnosis of chronic migraine and 2+ months of stable dose of pain medications</td>
<td>tDCS, target=L-DLPFC, anode=F3, cathode=Fp2. Active = 2 mA for 20 min, Sham = 2 mA for 30 s. 3 sessions per week for 4 weeks, for 12 total sessions. Audio guided mindfulness meditations during stimulation, and for at-home practice, 28 total days of mindfulness exercises.</td>
<td>FFMQ: Increases in both active and sham, with greater increases in active (d=1.1, p&lt;0.01) than sham (d=0.7, p=0.03). No significant interaction (d=0.2). Inability to perform daily living activities (MIDAS) showed improvements in both groups, with greater increases in active than sham</td>
<td>Seven total dropouts, three due to non-adherence to daily mindfulness exercises</td>
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<tr>
<td>Study</td>
<td>Methods</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcome Measures</td>
<td>Points of Interest</td>
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<tr>
<td>Pradhan &amp; Rossi, 2021</td>
<td>Combining Ketamine, Brain Stimulation (rTMS) and Mindfulness Therapy (TIMBER) for Opioid Addiction.</td>
<td>Open-label, proof-of-concept to test feasibility of ketamine + rTMS + TIMBER for opiate use disorder (OUD)</td>
<td>N=3. Adults with opiate use disorder (OUD)</td>
<td>rTMS, target=R-DLPFC. 10 Hz for 3000 pulses, 5 sessions over 1-2 weeks. TIMBER mindfulness-based therapy during same 2 weeks period + home practice twice daily. One week prior to rTMS and TIMBER, single infusion of 0.75 mg/kg ketamine</td>
<td>ASMI: 41.21% increase after treatment, (d=3.5, p=0.01)</td>
<td>Significant decrease in opiate craving (OCS)</td>
<td>TIMBER integrates all eight limbs of Yoga, five-factor model of trauma or addiction, and mindfulness-based graded exposure therapy (MB-GET). Balanced combination of both memory extinction and memory reconsolidation.</td>
</tr>
<tr>
<td>Study title</td>
<td>Sham-controlled, double-blind, randomized, clinical trial (DRKS00023490), to test feasibility of tDCS + mindfulness meditation for reducing pain in fibromyalgia patients</td>
<td>N=30, 2♂</td>
<td>28♀, age=53.6±1.7. 10 active + MM, 10 sham + MM, 10 no intervention. Adults diagnosed with fibromyalgia on stable treatment. Novice meditators.</td>
<td>tDCS, target=left motor cortex anode=C3, cathode=Fp2. Active = 2 mA for 20 min with 15 s ramp up and 15 s ramp down. Sham = 2 mA for 30 s ramp up and ramp down. 10 daily sessions over 2 weeks, simultaneous with audio guided mindfulness meditation. 5-minute body scan before 20-minute stim+meditation. 1 week of guided mindfulness meditation training before stimulation period.</td>
<td>FMI: No significant differences pre vs post in MM participants (d=0.82, p=0.082). No significant interaction between group and time (d=0.87, p=0.067)</td>
<td>Reduced pain intensity (PPT) in all three groups following therapy phase, no differences between groups. Significantly higher quality of life (FIQ) in active + MM group</td>
<td>Study run during COVID-19 pandemic, may have impacted patient response</td>
</tr>
</tbody>
</table>
Giorgi’s phenomenological method to investigate the lived experience of receiving rTMS for depression

N=9. Patients who completed the OPT-TMS depression treatment study

rTMS target=L-DLPFC. 10 Hz at 120% MT intensity for 3000 pulses.

5 sessions/week for 4–6 weeks

Mindfulness was an important theme for participants – the stimulation made them more mindful, and they believed their state of mind during stimulation affected outcomes

Narrative of frustration and helplessness, sensory experience of rTMS, and connection with the clinician were other major themes

Metacognitive themes in perception of treatment and self-observation were important to participants’ treatment response

<table>
<thead>
<tr>
<th>Citation</th>
<th>N</th>
<th>Sham</th>
<th>Participant health</th>
<th>NIBS type</th>
<th>Number of active NIBS sessions</th>
<th>Meditation during stimulation</th>
<th>Target</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abul Hasan et al., 2023</td>
<td>40</td>
<td>Yes</td>
<td>Healthy</td>
<td>tDCS</td>
<td>5, daily</td>
<td>Unspecified</td>
<td>Bilateral DLPFC</td>
<td>Time x group interaction: d=0.55*</td>
</tr>
<tr>
<td>Badran et al., 2016</td>
<td>12</td>
<td>Yes</td>
<td>Healthy</td>
<td>tDCS</td>
<td>2, weekly</td>
<td>Yes</td>
<td>R-DLPFC</td>
<td>Pre-post change: d=2.6</td>
</tr>
<tr>
<td>Brooks et al., 2021</td>
<td>26</td>
<td>Yes</td>
<td>Age&gt;60, symptoms of depression or anxiety + subjective cognitive complaints</td>
<td>tDCS</td>
<td>70 (variable adherence), daily</td>
<td>Yes</td>
<td>Bilateral DLPFC</td>
<td>Time x group interaction: d=0.6</td>
</tr>
<tr>
<td>Cavallero et al., 2021</td>
<td>27</td>
<td>No</td>
<td>Treatment-resistant MDD diagnosis</td>
<td>rTMS</td>
<td>35, daily</td>
<td>Yes</td>
<td>L-DLPFC</td>
<td>Pre-post change: d=0.612a</td>
</tr>
<tr>
<td>Danilewitz et al., 2021</td>
<td>22</td>
<td>Yes</td>
<td>Healthy</td>
<td>tDCS</td>
<td>1</td>
<td>Healthy</td>
<td>Bilateral DLPFC</td>
<td>Time x group interaction: d=0.132</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Treatment Parameters</td>
<td>tDCS</td>
<td>rTMS</td>
<td>MDE diagnosis</td>
<td>Time x group interaction:</td>
<td>Targeting and Stimulation Parameters</td>
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<tr>
<td>Gibson et al., 2022</td>
<td>Healthy adults wanting to reduce their drinking</td>
<td>tDCS</td>
<td>8, weekly</td>
<td>Yes</td>
<td>R-IFG</td>
<td>None</td>
<td>d=−0.1</td>
<td>Time x group interaction: d=−0.1</td>
</tr>
<tr>
<td>Hunter, 2016</td>
<td>Yes</td>
<td>Healthy</td>
<td>tDCS</td>
<td>1</td>
<td>Yes</td>
<td>R-IFG</td>
<td>None</td>
<td>Time x group interaction: d=−0.1</td>
</tr>
<tr>
<td>Leong et al., 2013</td>
<td>No</td>
<td>MDE diagnosis</td>
<td>rTMS</td>
<td>33±7</td>
<td>Unspecified</td>
<td>L- DLPFC</td>
<td>Pre-post change: d=1.3a</td>
<td>Time x group interaction: d=0.49</td>
</tr>
<tr>
<td>McCallion et al., 2020</td>
<td>Yes</td>
<td>Self-reported chronic pain</td>
<td>tDCS</td>
<td>8 (variable adherence), 2x/ week</td>
<td>Yes</td>
<td>DLPFC</td>
<td>Time x group interaction: d=0.2</td>
<td></td>
</tr>
<tr>
<td>Nishida et al., 2021</td>
<td>Yes</td>
<td>Healthy</td>
<td>tDCS</td>
<td>1</td>
<td>Yes</td>
<td>L- DLPFC</td>
<td>None</td>
<td>Time x group interaction: d=0.2</td>
</tr>
<tr>
<td>Pimenta et al., 2021</td>
<td>Yes</td>
<td>Chronic migraine</td>
<td>tDCS</td>
<td>12, 3x/week</td>
<td>Yes</td>
<td>L- DLPFC</td>
<td>Time x group interaction: d=0.2</td>
<td></td>
</tr>
<tr>
<td>Pradhan &amp; Rossi, 2021</td>
<td>No</td>
<td>Opiate use disorder</td>
<td>rTMS</td>
<td>5, 2-3x/week</td>
<td>Yes, but not during stim</td>
<td>R- DLPFC</td>
<td>Pre-post change: d=3.5</td>
<td></td>
</tr>
<tr>
<td>Ramasawmy et al., 2022</td>
<td>Yes</td>
<td>Fibromyalgia diagnosis</td>
<td>tDCS</td>
<td>10, daily</td>
<td>Yes</td>
<td>L-M1 motor cortex</td>
<td>Time x group interaction: d=0.87</td>
<td></td>
</tr>
<tr>
<td>Rosedale et al., 2009</td>
<td>No</td>
<td>MDD patients who completed OPT-TMS study</td>
<td>rTMS</td>
<td>20-30 (variable), daily</td>
<td>No</td>
<td>L- DLPFC</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

Note: * significant effect, a value is average of multiple effect sizes (see relevant entry in table 1).

**Targeting and Stimulation Parameters**

The most common target, found in 11 studies, was the DLPFC, with varied approaches.

The DLPFC is involved in executive functioning, task switching, information integration, and can inhibit nodes of the default mode network, making it a candidate target for excitation to
increase attentional control and self-regulation (Panikratova et al., 2020). Its position on the lateral surface of the frontal lobes makes it accessible for cortical stimulation. Three studies targeted bilateral DLPFC with tDCS: Abul Hasan et al. (2023) and Danilewitz et al. (2021) used an F3-F4 anode-cathode placement, while Brooks et al. (2021) used an Fz-Iz anode-cathode placement. Only Badran et al. (2017a) targeted R-DLPFC with tDCS, using a F8-supraorbital anode-cathode placement. Pradhan et al. (2020) targeted it with rTMS. Two studies targeted the L-DLPFC with tDCS: Nishida et al. (2021) used an F5-left shoulder anode-cathode placement, and Pimenta et al. (2021) used an F3-Fp2 anode-cathode placement. Three studies targeted L-DLPFC with rTMS: Cavallero et al. (2021), Leong et al. (2013), and Rosedale et al. (2009).

R-IFG was used as a target by Gibson et al. (2022) and Hunter (2016) using a F10-left upper arm anode-cathode placement. While DLPFC is involved in executive functioning more broadly, R-IFG is more specifically involved in impulse control, making it an appropriate target for treating addiction, such as in Gibson et al. (Gibson et al., 2022). Both studies found minimal mindfulness enhancement effects with their interventions. Ramasawmy et al. (2022) targeted left motor cortex with tDCS with a C3-Fp2 anode-cathode placement in fibromyalgia patients.

In tDCS, five studies used 30-minute sessions (Abul Hasan et al., 2023; Brooks et al., 2021; Gibson et al., 2022; Hunter, 2016; McCallion et al., 2020), and five studies used 20-minute sessions (Badran et al., 2017a; Danilewitz et al., 2021; Nishida et al., 2021; Pimenta et al., 2021; Ramasawmy et al., 2022). All studies used 2-mA except for Nishida et al., which used 1-mA, while Badran et al. had a 1-mA condition and a 2-mA condition. As previously described, the electrode placement was highly variable. While Nishida et al. showed minimal mindfulness effects, Badran et al. paradoxically showed significant increases in the Acting with Awareness subscale of the FFMQ only on the 1-mA condition and not the 2-mA.
All four TMS studies used rTMS at 10 Hz, which is a high-frequency stimulation frequency suitable for excitation. Cavallero et al. (2021), Leong et al. (2013), and Rosedale et al. (2009) targeted the L-DLPFC with 120%, 100%, and 120% of the motor threshold intensity, respectively, while Pradhan & Rossi (Pradhan et al., 2020) targeted the R-DLPFC with an unspecified power. All four studies administered stimulation for 3000 pulses or 30 minutes per session. This is a standard rTMS protocol commonly used as a treatment for depression that is comparable to intermittent theta burst stimulation (iTBS) in its effectiveness (Bakker et al., 2015). It has been shown to reduce rumination independent of its effects on depression, so it is a reasonable approach to improving mindfulness (Chu et al., 2023). Leong et al. (Leong et al., 2013) found that increases in decentering were independent of changes they found in depression.

**Measurement**

To measure mindfulness, five studies used the Five Factor Mindfulness Questionnaire (FFMQ) (Badran et al., 2017a; Cavallero et al., 2021; Leong et al., 2013; Nishida et al., 2021; Pimenta et al., 2021); three studies used the Mindful Attention Awareness Scale (MAAS) (Abul Hasan et al., 2023; Hunter, 2016; McCallion et al., 2020); three studies used the Toronto Mindfulness Scale (Badran et al., 2017b; Danilewitz et al., 2021; Gibson et al., 2022); one study used the Cognitive Affective Mindfulness Scale – Revised (Brooks et al., 2021); one study used the Experience Questionnaire (EQ) (Leong et al., 2013); one study used the Assessment Scale or Mindfulness Interventions (ASMI) (Pradhan et al., 2020); one study used the Freiburg Mindfulness Inventory (FMI) (Ramasawmy et al., 2022); and one study used Giorgi’s phenomenological method (Rosedale et al., 2009).

The Five Factor Mindfulness Questionnaire (FFMQ) is a widely used scale for broadly measuring trait mindfulness. Its items and five factors (observing, describing, acting with awareness, nonjudging of inner experience, and nonreactivity to inner experience) were
assembled from an analysis of existing mindfulness scales (Baer et al., 2008). Its trait-level items are engineered towards the “everyday” baseline features of mindfulness qualities, so it is to be expected that it would require a considerable intervention to significantly alter a person’s score. Five studies used it (Badran et al., 2017b; Cavallero et al., 2021; Leong et al., 2013; Nishida et al., 2021; Pimenta et al., 2021), and it showed the largest changes in two studies that treated depression with rTMS (Cavallero et al., 2021; Leong et al., 2013).

The Mindful Attention Awareness Scale (MAAS) is a single-dimension scale specifically focused on the presence or absence of attention to the present moment (Brown & Ryan, 2003). It is characterized by “mindlessness” questions that capture inattentiveness and “automatic pilot” behavior. Abul Hasan et al. (Abul Hasan et al., 2023) used the MAAS in their executive functioning enhancement trial. McCallion et al. (McCallion et al., 2020) used it in their chronic pain treatment. Both of these studies found significant differences between sham and active conditions, indicating their neuromodulation intervention reduced the “automatic pilot” behavior of their participants. Hunter (Hunter, 2016) used it with minimal effects detected.

While most other instruments are designed for trait-level mindfulness, the Toronto Mindfulness Scale is designed to capture state-level qualities of mindfulness. Its prompt that asks about “what you just experienced” is suitable for capturing acute experiences of mindfulness. Its two factors – decentering and curiosity – reflect an open, accepting state of awareness that is not trapped in overidentification. Danilewitz et al. (Danilewitz et al., 2021), Gibson et al. (Gibson et al., 2022), and Badran et al. (Badran et al., 2017b) all used it with minimal effects detected.

The Cognitive Affective Mindfulness Scale – Revised (CAMS-R) has four factors (attention, present-focus, awareness, and acceptance) (Feldman et al., 2007) and was used by Brooks et al. (Brooks et al., 2021) on their aging population study. The Experiences Questionnaire (EQ) is used as a measure of decentering (Fresco et al., 2007) and was used by
Leong et al. (Leong et al., 2013) as part of their depression treatment protocol. The Assessment Scale for Mindfulness Interventions (ASMI) is a proprietary scale used by Pradhan et al. (Pradhan, 2014; Pradhan et al., 2020). The Freiburg Mindfulness Inventory (FMI) was used by Ramasawmy et al. (Ramasawmy et al., 2022) to evaluate patient engagement in mindfulness practice. Finally, Rosedale et al. (Rosedale et al., 2009) employed Giorgi’s phenomenological method to investigate the lived experience of MDD patients receiving TMS.

**Intervention Protocols**

The intervention protocols were highly variable. To begin, three studies used only one active session (Danilewitz et al., 2021; Hunter, 2016; Nishida et al., 2021), all of which showed minimal mindfulness enhancement effects, though Hunter (Hunter, 2016) found working memory increases and a decoupling of DMN from focus-related processes, and Nishida et al. (Nishida et al., 2021) found reductions in anxiety scores that persisted for at least a week. Badran et al. (2017a) applied each 1-mA and 2-mA conditions once, one week apart, and found better state effects in the 1 mA than in the 2 mA condition. In general, the one-session protocols produced minimal improvements in mindfulness.

Gibson et al. (2022) used a weekly stimulation protocol over 8 weeks. Participants were given optional mindfulness-based relapse prevention (MBRP) lessons with which to engage at home, and they received weekly tDCS stimulation with guided meditation practice. They found minimal effects on mindfulness from their intervention, and their sham condition showed greater effectiveness in reducing alcohol cravings than the active condition. Their target was the R-IFG, also used by Hunter (2016), both of whom found minimal effectiveness.

McCallion et al. (2020), Pimenta et al. (2021), and Pradhan et al. (2020) performed stimulation 2-3 times a week. In McCallion et al., participants with chronic pain engaged in 30 minutes of breath-focused sitting meditation during tDCS stimulation of their DLPFC and then
received 1.5 hours of MBP instruction on formal practice, informal practice, and how to build a routine of practice. They found that participants who participated in more sessions had better outcomes in mindfulness and health functioning. Pimenta et al. (Pimenta et al., 2021) employed a tri-weekly L-DLPFC tDCS stimulation protocol on chronic migraine patients paired with daily at-home meditation lessons, and they found significant increases in mindfulness in both groups. The active group had greater increases than the sham group, though the time x stim group interaction effect was non-significant. Finally, Pradhan et al. (2020) administered 10-Hz rTMS to R-DLPFC 2-3 times a week on patients with OUD. Patients first received a single session of high-dose ketamine and then took part in TIMBER mindfulness therapy paired with a twice-daily meditation practice to treat their addiction. While they performed this therapy on only three patients, all three showed dramatic improvement in their symptoms and reported mindfulness.

Six studies used a daily stimulation protocol (Abul Hasan et al., 2023; Brooks et al., 2021; Cavallero et al., 2021; Leong et al., 2013; Ramasawmy et al., 2022; Rosedale et al., 2009). Three of these used tDCS (Abul Hasan et al., 2023; Brooks et al., 2021; Ramasawmy et al., 2022). Abul Hasan et al. (2023) did not perform any mindfulness instruction, focusing on increasing executive functioning with bilateral DLPFC stimulation. Increases in executive functioning would also be expected to increase scores on the MAAS (Lyvers et al., 2014). Brooks et al. (2021) provided their participants with a take-home tDCS kit to perform daily tDCS on their bilateral DLPFC for 10 weeks of MBSR instruction. Group instruction sessions were conducted weekly. While they did not find significant a time x stim group interaction on mindfulness, a moderate effect size was obtained (d=0.6, p=0.07). Additionally, they found trend improvements in anxiety, depression, social functioning, and cognitive functioning in their population of aging adults with clinical symptoms. Ramasawmy et al. (2022) applied daily tDCS to the left motor cortex of fibromyalgia patients simultaneously with mindfulness meditation
training. Similar to Brooks et al. (2021), their time x stim group interaction on mindfulness was just short of significant (p=0.067), likely due to low sample size, but their effect size was considerable (d=0.87).

The other three studies with a daily stimulation protocol used rTMS (Cavallero et al., 2021; Leong et al., 2013; Rosedale et al., 2009). Cavallero et al. (2021) employed 10-Hz rTMS to the L-DLPFC with simultaneous MBCT audio guidance on patients with treatment-resistant MDD for 5 weeks. They found significant improvements in mindfulness, depression symptoms, stress, quality of life, and interoception. However, they had a high attrition rate; some participants experienced discomfort from the rTMS, some had trouble trying to engage with the lessons during the stimulation period, and there were no improvements in their dropouts. Leong et al. (2013) performed a retrospective chart review of MDE patients who received a multi-week course of daily rTMS without any meditation training, finding increases in nonreactivity in the FFMQ and decentering in the EQ, along with improvements in depression and anxiety. They found that the increases in decentering were independent of improvements in depression. Finally, Rosedale et al. (2009) performed structured phenomenological interviews of MDD patients who completed a course of rTMS, and they found that the stimulation made patients more mindful, and that these increases in mindfulness and other aspects of metacognition played an important role in the outcomes of their treatment. It is worth noting that both Leong et al. and Rosedale et al. did not employ any mindfulness training, but they still found mindfulness effects, suggesting the course of stimulation therapy alone was responsible.

Altogether, it appears that more intensive approaches that use daily training and regular, repeated stimulation showed more success than more limited interventions.
Discussion

We have outlined 14 studies from the literature that connected mindfulness outcomes with NIBS. The most common approach was to stimulate DLPFC to enhance cognitive control. Some studies showed significant improvement compared to their sham stimulation conditions, while others showed no such improvement. From these results, we can conclude that NIBS interventions are promising techniques to enhance mindfulness training and its outcomes, but the consistency and reliability of these techniques have not yet been demonstrated. Clearly, more work is needed to develop a consensus on stimulation parameters and target location that would enable an empirically-supported paradigm. Several issues need to be addressed before mindfulness enhancement with NIBS can advance.

First, NIBS techniques must become more sophisticated and fine-grained. Existing techniques of tDCS lack spatial specificity, and it is questionable how well they can target areas like DLPFC (Soleimani et al., 2023). The lack of focal specificity makes it hard to draw clear conclusions about which neural targets are contributing to the effects, or to create reliable effects across participants and sessions. High-definition tDCS improves precision, but its specificity is still limited (Edwards et al., 2013). TMS is more spatially specific, but it cannot penetrate the brain beyond the outer cortex, limiting its range of application (Klomjai et al., 2015). Emerging tools like tFUS or temporal interference (TI) can target deep brain sites with high accuracy (Blackmore et al., 2019; Grossman, 2008; Violante et al., 2023), allowing for clearer conclusions to be drawn about the underlying neural circuitry that is being targeted.

Second, as the NIBS tools get more fine-grained, the psychological constructs must also be sharpened. ‘Mindfulness’ functions as an umbrella term for both the contemplative practices and the qualities of mind that they evoke. However, its indiscriminate meaning causes numerous survey instruments to capture very different things. In the context of meditation training, it may
be more accurate and valuable to describe skills that are being refined rather than states being experienced or inherent traits being changed over time with effortful practice. While the state of mindfulness is important, as participants have direct access to it, we argue it is more useful to think of mindfulness as a collection of focus skills that can be deliberately cultivated with systematic training. We can consider a baseline level of each skill in the way we measure a person’s baseline level of physical strength, which can be dramatically elevated with a well-organized regimen of physical exercise. For example, we have defined core skills as concentration, clarity, and equanimity (Young, 2016). Systematic mindfulness practice elevates each of these skills, which enhances their awareness and capacity to be present with ongoing experience with an attitude of openness and acceptance (i.e., equanimity). Measurement instruments that reflect the skilled nature of mindfulness, such as a stress test (equanimity) (Lindsay, Young, et al., 2018), physical stillness index (concentration) (Kang-Ming Chang et al., 2016), or a neural stability signature (also concentration) (Bailey et al., 2024) may reflect critical aspects of mindfulness skills that complement psychometric instruments. Measuring the baseline level of each skill allows NIBS researchers to target circuits in the brain relevant for each skill, in the way an exercise machine may target different muscle groups. Focusing on specific skills also allows researchers to connect them with more specific psychiatric symptoms (e.g., rumination or panic). Correspondingly, a lack of understanding or focus on specific skills risks dampening the effectiveness of a given mindfulness training regimen.

One of the only clear and dominant trends of this review was the approach of targeting DLPFC to stimulate the cognitive control system. This approach focuses exclusively on the attentional process aspect of mindfulness (or “concentration” in the Young model) (Young, 2016). Attention and cognitive control are part of mindfulness, but mindfulness is not entirely contained within these processes. It is unlikely that enhancing cognitive control processes alone
leads to the same widespread beneficial outcomes as seen from mindfulness practice. There is an intermediate step that must be clarified between the training and the acquisition of the skills. More bluntly, if cognitive control can be enhanced, what is it being enhanced for?

An important mindfulness skill beyond the attentional lens is equanimity, which has been defined as the capacity to be open and accepting towards present experience irrespective of the emotional valence (positive, negative, or neutral). Lindsay et al. (2018; 2018; 2019) used a dismantling trial to show that equanimity practice combined with concentration training (as compared to concentration or sham mindfulness alone) was a key ingredient to improved outcomes in positive emotions, stress reactivity, and loneliness. This piece of the mindfulness puzzle may be the keystone to what makes mindfulness so different and more beneficial than simple cognitive training or stress reduction. Therefore, it may be more advantageous to enhance equanimity with NIBS rather than cognitive control processes. Recent work from our group (Lord et al., submitted) followed such an approach, where we hypothesized that targeting brain structures that may be related to “getting caught up” in experience (J. Brewer et al., 2013) would enhance state equanimity. We are now testing the hypothesis that enhanced state equanimity will facilitate systematic mindfulness training that targets not only equanimity but also concentration and clarity. Thus, enhanced equanimity should generally enhance mindfulness training. These results were recently replicated by an independent lab, Cain et al. (Cain et al., 2024).

In sum, for an empirically-supported mindfulness enhancement paradigm to emerge, the field needs to get more fine-tuned in all aspects of its approach, including the stimulation, the psychological constructs, and the mindfulness training. Ultimately, the goal of these NIBS interventions is not to take over the effort of building skills but to accelerate and enhance skill-building so that in the absence of stimulation, the person still has the skills to cope with stress and is more resilient to developing psychiatric symptoms. A metaphor that may be appropriate is
that the neuromodulation could provide “training wheels” for the brain to carve out the necessary neural pathways.

**Conclusion**

Given the positive effects of mindfulness practice, it is reasonable to pursue a regime using NIBS that can enhance the acquisition of mindfulness skills and their benefits. However, the fields of mindfulness and brain stimulation both need to refine their techniques and become more fine-grained in their approach to develop more consistent and effective methods. Ultimately, better methods to promote skill-building in mindfulness would have therapeutic and preventative benefits for those with psychiatric disorders and help us better understand the neural mechanisms of mindfulness.
Acknowledgments

CRediT author statement: **Brian Lord**: Conceptualization, Methodology, Investigation, Writing - Original Draft. **John Allen**: Writing – Review & Editing, Supervision. **Shinzen Young**: Conceptualization, Writing – Review & Editing. **Jay Sanguinetti**: Conceptualization, Writing – Review & Editing, Supervision. Some of the ideas within this paper were inspired at the M4 Conference in St Louis, MO in 2023.
Disclosures

Dr. Sanguinetti is paid a salary and is a shareholder in Sanmai Technologies, PBC. Dr. Young is paid a consulting fee from Sanmai Technologies, PBC. Dr. Allen and Lord have no conflicts of interest to declare.
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https://doi.org/10.1177/1078390309350773


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Appendix B: Transcranial Focused Ultrasound to the Posterior Cingulate Cortex Modulates Default Mode Network: A Pilot Study

Transcranial Focused Ultrasound to the Posterior Cingulate Cortex Modulates Default Mode Network: A Pilot Study

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Keywords: transcranial focused ultrasound, neuromodulation, non-invasive brain stimulation, default mode network, mindfulness
Abstract

Background: Transcranial focused ultrasound (TFUS) is an emerging neuromodulation tool for temporarily altering brain activity and probing network functioning. The effects of TFUS on the default mode network (DMN) are unknown.

Objective: The study examined the effects of transcranial focused ultrasound (TFUS) on the functional connectivity of the default mode network (DMN), specifically by targeting the posterior cingulate cortex (PCC). Additionally, we investigated the subjective effects of TFUS on mood, mindfulness, and self-related processing.

Methods: The study employed a randomized, single-blind design involving thirty healthy subjects. Participants were randomly assigned to either the active TFUS group or the sham TFUS group. Resting-state functional magnetic resonance imaging (rs-fMRI) scans were conducted before and after the TFUS application. To measure subjective effects, the Toronto Mindfulness Scale, the Visual Analog Mood Scale, and the Amsterdam Resting State Questionnaire were administered at baseline and 30 minutes after sonication. The Self Scale and an unstructured interview were also administered 30 minutes after sonication.

Results: The active TFUS group exhibited significant reductions in functional connectivity along the midline of the DMN, while the sham TFUS group showed no changes. The active TFUS group demonstrated increased state mindfulness, reduced Global Vigor, and temporary alterations in the sense of ego, sense of time, and recollection of memories. The sham TFUS group showed an increase in state mindfulness, too, with no other subjective effects.

Conclusions: TFUS targeted at the PCC can alter DMN connectivity and cause changes in subjective experience. These findings support the potential of TFUS to serve both as a research tool and as a potential therapeutic intervention.
Introduction

The default mode network (DMN) has been the subject of extensive research since it was initially defined by Raichle et al. (2001). The DMN is anchored by two midline nodes at the medial prefrontal cortex (mPFC) and the posterior cingulate cortex (PCC) with adjacent bilateral nodes in the angular gyri and middle temporal gyri (Andrews-Hanna et al., 2010). While theoretical ideas and debate about the function of the DMN continue to evolve (Andrews-Hanna et al., 2014a; Yeshurun et al., 2021), it appears to play an essential role in the inner processes of mind-wandering, planning, and self-related processing (Raichle, 2015).

The DMN has been implicated in several disorders, including depression (Ju et al., 2022; Scalabrini et al., 2020; Zhou et al., 2020), addiction (Zhang and Volkow, 2019), autism (Padmanabhan et al., 2017), ADHD (Harikumar et al., 2021; Posner et al., 2014), and schizophrenia (Hu et al., 2017). Collectively, the clinical literature suggests that a breakdown in the regulation of the DMN may be one mechanism underlying these disorders (e.g., abnormal DMN activity leading to increased rumination in depression) (Lydon-Staley et al., 2019; Zhou et al., 2023). Thus, potential treatments that normalize DMN function may be called for (Holtzheimer and Mayberg, 2011; Scalabrini et al., 2020).

Empirical research with psychedelics has implicated the DMN in constructing varying states of consciousness and representations of the self, especially the ‘narrative self,’ in which the self is the object of thought (Nour and Carhart-Harris, 2017). The current scientific consensus establishes that disruption of resting state functional connectivity within the DMN is a central mechanism that drives their profound psychological and therapeutic effects (Carhart-Harris et al., 2012; Gattuso et al., 2022). Carhart-Harris and colleagues (2012) found that the degree to which psilocybin decreased cerebral blood flow and connectivity of the mPFC and PCC predicted the magnitude of the self-altering effects.
The practice of meditation can also alter resting-state brain activity, including the DMN. Brewer and colleagues (2013) found reduced DMN connectivity in experienced meditators during meditation in the MRI scanner. The same group demonstrated that meditators could volitionally reduce activity in their PCC using real-time fMRI neurofeedback, and that this reduction correlated with their internal meditative experience (Garrison et al., 2013).

Neurophenomenological analysis suggested that PCC deactivation was associated with an experience of “undistracted awareness” and “effortless doing” (Garrison et al., 2013). This led Brewer and colleagues (Brewer et al., 2013; Brewer and Garrison, 2014) to suggest that PCC activity plays a role in self-referential processing, particularly a tendency to “get caught up in” one’s experience. In the mindfulness literature, this quality may also be described as a lack of equanimity (Desbordes et al., 2015). Importantly, mindfulness training, as demonstrated by Korponay and colleagues (2019), does not simply reduce DMN activity but rather enhances one’s ability to control and inhibit it when necessary.

The extant fMRI literature in healthy individuals, clinical studies, psychedelics, and contemplative experiments all converge on the major role of the DMN in internal thought and self-related processing (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014b; Carhart-Harris and Friston, 2019; Davey et al., 2016; Davey and Harrison, 2022; Nour and Carhart-Harris, 2017). Non-invasive brain stimulation techniques that enable targeted manipulation of DMN regions would offer an opportunity to estimate the causal relationship between DMN activity and internal processes, paving the way to potential effective therapeutics.

A promising method for non-invasive brain modulation is transcranial focused ultrasound (TFUS). TFUS modulates brain regions with pulsed beams of focused ultrasound with millimeter precision (Blackmore et al., 2019). Unlike other non-invasive brain stimulation techniques like transcranial electrical stimulation (TES) or transcranial magnetic stimulation
(TMS), TFUS can effectively reach deep subcortical regions like the thalamus (Kim et al., 2023; Legon et al., 2018) by adjusting the focal depth of the ultrasound beam. The safety profile of TFUS is favorable: the current picture is that adverse events only occur when stimulation is too long and/or intense, far in excess of FDA safety limits, causing thermal and/or biophysical damage to the targeted tissue or unintentional opening of the blood-brain barrier (Pasquinelli et al., 2019).

TFUS acts through a combination of potential mechanisms, including thermal, mechanical, and cavitation effects as a result of the acoustic energy interacting with neural tissue (Dell’Italia et al., 2022). Reviews suggest that it can be both excitatory and inhibitory in its effects (Zhang et al., 2021), but there is not always a clear differentiation between the two, as some studies show both excitatory and inhibitory effects simultaneously (Verhagen et al., 2019; Yoon et al., 2019), while others show state-dependent (Yang et al., 2021) or cell type-dependent (Wattiez et al., 2017) responses. Given that similar effects can arise from bidirectional mechanisms (e.g., inhibition of excitatory neurons or stimulation of inhibitory neurons can both produce suppression), it is more accurate to describe the neuromodulatory effects at the tissue-level. Network (Folloni et al., 2019) and distal (Cain et al., 2021) effects can also manifest, as can delayed, offline effects (Carhart-Harris et al., 2012). Sanguinetti and colleagues (2020) found that targeting the right prefrontal cortex induced mood enhancement and decreased functional connectivity in regions distal from the target.

Given these capabilities, TFUS is an ideal candidate for non-invasively modulating the DMN. The aim of this pilot study was to target the PCC using TFUS parameters that are expected to suppress neural firing (Dell’Italia et al., 2022), specifically by utilizing a low duty cycle value of 5.26%. The hypothesis was that this approach would enable modulation of the resting state connectivity from that node to the rest of the network. We also hypothesized that
this would induce changes in phenomenology that relate to DMN activity, specifically mindfulness and self-referential processing. As a proof-of-concept, this would pave the way for TFUS to serve as a tool to probe network functioning and be used as a therapeutic intervention.

**Materials & Methods**

**Subjects**

Thirty healthy subjects (18 female, average age 19.8 years) participated in this study. Exclusion criteria were: use of tobacco/nicotine, history of head injury, uncorrected hearing or vision impairment, history of brain or mental illness (including drug and/or alcohol dependence), use of pharmaceuticals (including psychotropic drugs), sleep disorders, drug or alcohol intoxication, history of epilepsy, history of migraines, metal implants in their head, and history of cardiac problems. Inclusion criteria were: age 18-77, normal or corrected vision, and proficient enough in English to read the consent form. Subjects received either actual or sham TFUS in a single-blind, between-subjects design.

**Procedure**

After screening and consent, subjects were given subjective rating scales. Subsequently, they underwent four MRI scans: T1 structural, baseline functional resting state (rs-BOLD), arterial spin labeling (rs-pcASL), and susceptibility weighted imaging (SWI). During functional scans, subjects were instructed to stare at a fixation cross and allow their thoughts to flow naturally. Subjects were removed from the scanner, and real-time neuronavigation (Visor2, ANT Neuro, the Netherlands) was used to apply either active or sham TFUS to their ventral PCC based on their individual structural MRI. Sham TFUS was performed by holding an unplugged transducer against their head. Subjects then returned to the MRI scanner for further measurements. Functional MRI scans were captured in the 5 minutes immediately after application (t1) and at 25 minutes after application (t2) (see Figure 1). Post-sonication SWI and
pcASL scans were also taken after t1. Upon exiting the scanner, final subjective rating scales were taken, and the subject was debriefed.

**Figure 1**

*Timeline of MRI Data Acquisition and TFUS Application*

**Subjective Ratings**

Before any MRI scans, subjects responded to the Visual Analog Mood Scale (VAMS) (Luria, 1975) and the Toronto Mindfulness Scale (TMS) (Lau et al., 2006). After TFUS and all subsequent MRI scans, they responded to the same scales again, along with the Self Scale (Lebedev et al., 2015) and the Amsterdam Resting-State Questionnaire (ARSQ) (Diaz et al., 2013).

**Post Experiment Questions**

Structured post-experiment questions were asked of each participant. Participants were asked to guess if they were in the “stimulation or placebo condition,” whether the ultrasound changed their “overall mental state,” if they heard anything from the transducer, and if they had changes to their “inner talk- or thinking-space.”

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Figure 1. Data were gathered before and after TFUS application.

**tfUS application**

<table>
<thead>
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<th>baseline resting state scan (t₀)</th>
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| target: PCC AF: 500 kHz 2500 cycles/pulse PRF: 10.526 Hz DC: 5.26% 30s on, 30s off 5 min total or sham tfUS application: transducer held to head with no energy emitted |

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<table>
<thead>
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<th>+25 min resting state (t₂)</th>
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<td>rs-BOLD (6 min)</td>
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</table>
MRI scans

Functional BOLD images were acquired on a Siemens Skyra 3-Tesla scanner using EPI gradient echo sequence (TR = 1800 ms; TE = 25 ms; flip angle = 90; FOV = 192 mm; acquisition voxel size 3 mm x 3 mm x 3 mm). T1-weighted anatomical images were also acquired for neuronavigation and registration of the functional scans (MP-RAGE; TR = 2500 ms; TE = 4.35 ms; TI = 900 ms; flip angle = 8; FOV = 256 mm).

Acoustic Intensity Measurements

Acoustic intensity was measured using a custom-built water tank setup. Data were recorded using a needle hydrophone (HNR-0500; Onda, Sunnyvale, CA, USA) with a geometric diameter of 2.5 mm. A scan volume of 12mm (x), 12mm (y), 68mm (axial) was collected using .508 mm steps in degassed water. Pressure and intensity were calculated from the voltage recordings from the hydrophone. MI was calculated with a derated peak pressure using the attenuation coefficient of soft tissue (.3dB/cm) (Abbott, 1999). The measured output of this wave in degassed free water shows a peak negative pressure of 0.422 MPa. The output of the beam through a hydrated sample of cadaver parietal bone showed a peak negative pressure of 0.130 MPa (a 69.3% decrease). See Figure 2. Full properties of the wave output are presented in Table 1. The skull caused little deviation of the focus in the lateral plane, but in the axial plane focus became more shallow (axial focus water = 56.6 mm; skull = 51.7mm; difference = -4.8 mm). See Table 2. The sawtooth pattern in the axial plane (panel C of figure 2) could be due to an artifact in the hydrophone setup (caused by standing waves or reflections created by the four radial elements).

Figure 2

Acoustic Intensity Measurements
Figure 2. A. Skull attenuation and geometric deformation of acoustic temporal peak pressure recorded in water and through a sample of cadaver parietal bone (‘skull’). Center of the beam and FWHM are displayed. Lateral shift displayed in A and axial shift in C. Sawtooth pattern in panel C ) could be due to an artifact in the hydrophone setup (caused by standing waves or reflections created by the four radial elements). B. Skull-attenuated ultrasound intensity map is overlaid on a single subject’s MRI. Estimation of peak focus was determined using recorded neuronavigation coordinates from that subject’s TFUS session. D. Spatial distribution of temporal peak intensity of the ultrasound beam in water (top) and through a cadaver parietal skull piece (bottom). Left panel shows lateral spatial topography of temporal peak intensity of the beam at the axial peak; right panel displays axial topography.
Table 1

**Acoustic Wave Analysis**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Water</th>
<th>Skull</th>
<th>Units</th>
<th>% decrease through skull</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISPTP</td>
<td>11.91</td>
<td>1.13</td>
<td>W/cm²</td>
<td>90.5%</td>
<td>Spatial peak temporal peak intensity</td>
</tr>
<tr>
<td>ISPPA</td>
<td>5.58</td>
<td>0.46</td>
<td>W/cm²</td>
<td>91.8%</td>
<td>Spatial peak pulse average intensity</td>
</tr>
<tr>
<td>ISPTA</td>
<td>293.37</td>
<td>23.98</td>
<td>mW/cm²</td>
<td>91.8%</td>
<td>Spatial peak temporal average intensity</td>
</tr>
<tr>
<td>PSPTP</td>
<td>421.57</td>
<td>129.60</td>
<td>kPa</td>
<td>69.3%</td>
<td>Spatial peak temporal peak pressure</td>
</tr>
<tr>
<td>PSPPA</td>
<td>260.52</td>
<td>73.86</td>
<td>kPa</td>
<td>71.7%</td>
<td>Spatial peak pulse average pressure</td>
</tr>
<tr>
<td>PSPTA</td>
<td>13.70</td>
<td>3.88</td>
<td>kPa</td>
<td>71.6%</td>
<td>Spatial peak temporal average pressure</td>
</tr>
<tr>
<td>MI*</td>
<td>0.60</td>
<td>0.18</td>
<td>-</td>
<td>69.3%</td>
<td>Mechanical index</td>
</tr>
</tbody>
</table>

*Note: *MI derated for soft tissue from water only*

Table 2

**Skull Distortion Measurement**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Axial</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Skull</td>
</tr>
<tr>
<td>Focal peak</td>
<td>56.5 mm</td>
<td>51.7 mm</td>
</tr>
<tr>
<td>Intensity FWHM start</td>
<td>45.6 mm</td>
<td>42.3 mm</td>
</tr>
<tr>
<td>Intensity FWHM end</td>
<td>71.4 mm</td>
<td>69.4 mm</td>
</tr>
<tr>
<td>Intensity FWHM length</td>
<td>25.9 mm</td>
<td>27.1 mm</td>
</tr>
<tr>
<td>Power FWHM start</td>
<td>40.6 mm</td>
<td>30.2 mm</td>
</tr>
<tr>
<td>Power FWHM end</td>
<td>81.9 mm</td>
<td>78.4 mm</td>
</tr>
<tr>
<td>Power FWHM length</td>
<td>41.3 mm</td>
<td>48.3 mm</td>
</tr>
</tbody>
</table>

**TFUS Stimulation**

Subjects were seated comfortably. An MRI-guided stereotactic system (Visor2, ANT Neuro, the Netherlands) was used to guide TFUS targeting to the participant’s PCC. The focused ultrasound was delivered by a custom 4-channel ring transducer (Sonic Concepts, Bothell, WA,
USA) with an outer diameter of 64 mm that uses a sealed membrane filled with degassed water for coupling, which is then housed inside a custom 3D-printed casing. The transducer was driven by an acoustic amplifier (TPO-203, Sonic Concepts, Bothell, WA, USA), with the ultrasound beam having the following parameters: acoustic frequency (AF) = 500 kHz, pulse repetition frequency (PRF) = 10.526 Hz, pulse repetition period (PRP) = 95 ms, pulse duration (PD) = 5 ms or 2500 cycles, duty cycle = 5.26%. Badran and colleagues used similar parameters to suppress the pain pathway, except they used a 650 kHz acoustic frequency (Badran et al., 2020; Li et al., 2021).

The beam was focused to fixed distance of 55 mm, corresponding to the average distance of the PCC from the surface of the scalp where the transducer is applied. The target was determined by inspection of each subject’s anatomical scan. The ventral PCC was chosen due to its greater association with internal directed thought rather than the cognitive control functions associated with the dorsal PCC (Leech and Sharp, 2014).

The subject’s head was registered to their structural MRI in the Visor2 neuronavigation software using three fiducials (nasion and ears) and their scalp surface. The transducer was held firmly against the subject’s head, using individual MR-guided neuronavigation, with gel applied to the scalp, to deliver 30-second stimulus intervals followed by 30-second rest periods. The pattern of [30s ON, 30s OFF] was repeated five times consecutively, totaling a 5-minute duration. Participants were instructed to sit quietly with their eyes open. The researcher periodically (about every 1.5 minutes) asked them how they were doing, if they felt anything unusual, and if they would like to continue.
MRI Analysis

Results included in this manuscript come from analyses performed using CONN (Whitfield-Gabrieli and Nieto-Castanon, 2012) (RRID:SCR_009550) release 21.a (Nieto-Castanon and Whitfield-Gabrieli, 2021) and SPM (Penny et al., 2011) (RRID:SCR_007037) release 12.7771.

Preprocessing

Functional and anatomical data were preprocessed using a flexible preprocessing pipeline (Nieto-Castanon, 2020a) including realignment with correction of susceptibility distortion interactions, slice timing correction, outlier detection, direct segmentation and MNI-space normalization, and smoothing. Functional data were realigned using SPM realign & unwarp procedure (Andersson et al., 2001), where all scans were coregistered to a reference image (first scan of the first session) using a least squares approach and a 6 parameter (rigid body) transformation (Friston et al., 1995), and resampled using b-spline interpolation to correct for motion and magnetic susceptibility interactions. Temporal misalignment between different slices of the functional data (acquired in interleaved Siemens order) was corrected following SPM slice-timing correction (STC) procedure (Henson et al., 1999; Sladky et al., 2011), using sinc temporal interpolation to resample each slice BOLD timeseries to a common mid-acquisition time. Potential outlier scans were identified using ART (Whitfield-Gabrieli et al., 2011) as acquisitions with framewise displacement above 0.9 mm or global BOLD signal changes above 5 standard deviations (Nieto-Castanon, submitted; Power et al., 2014), and a reference BOLD image was computed for each subject by averaging all scans excluding outliers. Functional and anatomical data were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and resampled to 2 mm isotropic voxels following a direct normalization procedure (Calhoun et al., 2017; Nieto-Castanon, submitted) using SPM unified
segmentation and normalization algorithm (Ashburner, 2007; Ashburner and Friston, 2005) with the default IXI-549 tissue probability map template. Last, functional data were smoothed using spatial convolution with a Gaussian kernel of 8 mm full width half maximum (FWHM).

**Denoising**

In addition, functional data were denoised using a standard denoising pipeline (Nieto-Castanon, 2020b) including the regression of potential confounding effects characterized by white matter timeseries (5 CompCor noise components), CSF timeseries (5 CompCor noise components), motion parameters and their first order derivatives (12 factors) (Friston et al., 1996), outlier scans (below 35 factors) (Power et al., 2014), session and task effects and their first order derivatives (6 factors), and linear trends (2 factors) within each functional run, followed by bandpass frequency filtering of the BOLD timeseries (Hallquist et al., 2013) between 0.008 Hz and 0.09 Hz. CompCor (Behzadi et al., 2007; Chai et al., 2012) noise components within white matter and CSF were estimated by computing the average BOLD signal as well as the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject’s eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to range from 130.9 to 147.6 (average 144.5) across all subjects (Nieto-Castanon, submitted).

**First-level analysis**

ROI-to-ROI connectivity (RRC) matrices were estimated characterizing the functional connectivity between each pair of regions among 100 ROIs (Schaefer et al., 2018). Functional connectivity strength was represented by Fisher-transformed bivariate correlation coefficients from a general linear model (weighted-GLM) (Nieto-Castanon, 2020c), estimated separately for each pair of ROIs, characterizing the association between their BOLD signal timeseries.
Individual scans were weighted by a boxcar signal characterizing each individual task or experimental condition convolved with an SPM canonical hemodynamic response function and rectified.

**Group-level analyses**

Group-level analyses were performed using a General Linear Model (GLM) (Nieto-Castanon, 2020d). A contrast that averages both post-stimulation conditions against the baseline (t_1+t_2>baseline) was designed to estimate a general model of the effects of tFUS for each active and sham group. Time points (t_1 and t_2) were combined to enhance the statistical power of the model due to the small sample size. Individual time point models were also estimated for each condition (t_1>baseline and t_2>baseline) in an exploratory secondary analysis to examine the temporal nature of the effects. For each individual connection a separate GLM was estimated, with first-level connectivity measures at this connection as dependent variables (one independent sample per subject and one measurement per task or experimental condition, if applicable), and groups or other subject-level identifiers as independent variables. Connection-level hypotheses were evaluated using multivariate parametric statistics with random-effects across subjects and sample covariance estimation across multiple measurements. Inferences were performed at the level of individual clusters (groups of contiguous connections). Cluster-level inferences were based on nonparametric statistics using Threshold Free Cluster Enhancement (TFCE) (Smith and Nichols, 2009), with 1000 residual-randomization iterations, and ROIs sorted using optimal leaf ordering based on ROI-to-ROI anatomical proximity and functional similarity metrics (Bar-Joseph et al., 2001; Nieto-Castanon, 2020e). For the primary effects model (t_1+t_2>baseline), results were conservatively thresholded using a combination of a cluster-forming p<0.001 connection-level threshold and a familywise corrected p-FDR<0.01 cluster-mass threshold. For
the secondary exploratory analysis of individual time points, less conservative thresholds were used (connection-level p<0.05 and cluster-level p<0.05).

Results

Functional Connectivity

ROI-to-ROI analysis revealed significant decreases in connectivity in the active group within 1 cluster comprising 8 ROIs and 11 connections between them, while there were no significant changes in the sham group (see Table 3). The cluster comprised a decrease in connectivity between midline nodes of the DMN. There were reductions in connectivity across hemispheres in the cingulate cortex, and the medial and dorsolateral prefrontal cortex reduced in connectivity with the posterior cingulate (see Figure 3). The same model was estimated for the sham group with no significant effects found. While a single model that contrasted active and sham groups found no significant effects, the effects found in the active model were highly significant after conservative corrections were made.

Table 3

ROI-to-ROI Functional Connectivity Analysis of Effects of TFUS

<table>
<thead>
<tr>
<th>Analysis Unit</th>
<th>Statistic</th>
<th>p-unc</th>
<th>p-FDR</th>
<th>p-FWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>Score = 209.67</td>
<td>0.0008</td>
<td>0.0237</td>
<td>0.0050</td>
</tr>
<tr>
<td></td>
<td>Mass = 576.39</td>
<td>0.0001</td>
<td>0.0048</td>
<td>0.0010</td>
</tr>
<tr>
<td></td>
<td>Size = 22</td>
<td>0.0001</td>
<td>0.0038</td>
<td>0.0010</td>
</tr>
<tr>
<td>Right Cingulate Posterior – Left Cingulate Posterior</td>
<td>T(14) = -7.20</td>
<td>&lt;0.0001</td>
<td>0.0159</td>
<td></td>
</tr>
<tr>
<td>Left Cingulate Posterior – Right Precuneus Posterior Cingulate</td>
<td>T(14) = -5.67</td>
<td>&lt;0.0001</td>
<td>0.0507</td>
<td></td>
</tr>
<tr>
<td>Left Cingulate Posterior – Right Medial Prefrontal Cortex</td>
<td>T(14) = -5.53</td>
<td>0.0001</td>
<td>0.0507</td>
<td></td>
</tr>
<tr>
<td>Right Cingulate Posterior – Right Medial Prefrontal Cortex</td>
<td>T(14) = -5.18</td>
<td>0.0001</td>
<td>0.0555</td>
<td></td>
</tr>
<tr>
<td>Left Cingulate Posterior – Right Dorsal Prefrontal Cortex</td>
<td>T(14) = -5.05</td>
<td>0.0002</td>
<td>0.0604</td>
<td></td>
</tr>
<tr>
<td>Right Cingulate Posterior – Right Precuneus Posterior Cingulate</td>
<td>T(14) = -4.79</td>
<td>0.0003</td>
<td>0.0808</td>
<td></td>
</tr>
<tr>
<td>Comparisons</td>
<td>T(14)</td>
<td>p-unc</td>
<td>p-FDR</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Right Cingulate Posterior – Left Precuneus</td>
<td>-4.71</td>
<td>0.0003</td>
<td>0.0882</td>
<td></td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Cingulate Posterior – Left Precuneus</td>
<td>-4.51</td>
<td>0.0005</td>
<td>0.0989</td>
<td></td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Precuneus Posterior Cingulate – Left</td>
<td>-4.40</td>
<td>0.0006</td>
<td>0.0989</td>
<td></td>
</tr>
<tr>
<td>Precuneus Posterior Cingulate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Cingulate Posterior – Left Dorsal</td>
<td>-4.35</td>
<td>0.0007</td>
<td>0.0989</td>
<td></td>
</tr>
<tr>
<td>Prefrontal Cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Cingulate Posterior – Left Medial</td>
<td>-4.19</td>
<td>0.0009</td>
<td>0.1084</td>
<td></td>
</tr>
<tr>
<td>Prefrontal Cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* p-unc = uncorrected p-value. p-FDR = false discovery rate corrected p-value. p-FWE = family wise error rate corrected p-value.
Figure 3

ROI-to-ROI Functional Connectivity Changes from Baseline to Average of $t_1$ and $t_2$

Figure 3. A. Sagittal, coronal, and axial views of significant ROI-to-ROI functional connectivity changes in active TFUS condition, all of which were decreases in functional connectivity (represented by blue connecting lines, with the affected ROIs highlighted in yellow) within and along the midline of the DMN and cingulate cortex. B. No significant ROI-to-ROI functional connectivity changes found in the sham TFUS condition.
Models were estimated for the effects at individual time points ($t_1$>baseline and $t_2$>baseline) within each condition. In $t_1$ of the active condition, reductions in connectivity were seen along the midline of the DMN within 1 cluster comprising 7 ROIs and 11 connections between them (see table S1 for details). In $t_2$, these effects are more diffuse, extending to the left and right inferior parietal lobes, the left and right medial parietal lobes, and the left temporal pole, within 1 cluster comprising 17 ROIs and 45 connections between them (see table S2 for details). See panels A and B in figure 4.

In the sham condition, connectivity reductions were seen in $t_1$ primarily within somatomotor and dorsal attention networks, within 1 cluster comprising 26 ROIs and 69 connections between them (see table S3 for details). No effects were seen in $t_2$. See panels C and D in figure 4.

**Figure 4**

*ROI-to-ROI Functional Connectivity Changes from Baseline to $t_1$ and $t_2$ in Active Condition*

*Figure 4.* Sagittal and axial views of significant ROI-to-ROI functional connectivity changes in each timepoint compared to baseline for each condition, all of which were decreases in functional connectivity (represented by blue connecting lines, with the affected ROIs
highlighted in yellow). A. Contrast of t₁ to baseline in active condition. B. Contrast of t₂ to baseline in active condition. C. Contrast of t₁ to baseline in sham condition. D. Contrast of t₂ to baseline in sham condition.

Subjective Ratings

Psychometric Scales

Due to small sample sizes (n=15 for each condition) and skewed results in many test questions, we opted for non-parametric tests (Wilcoxon rank sum tests and linear mixed models) instead of parametric tests to analyze the psychometric scales. The Wilcoxon rank-sum test compares ranks instead of raw data, and linear mixed models are less sensitive to violations of normality. These tests are more robust, less affected by outliers or skewed data, reducing the risk of drawing incorrect conclusions.

Toronto Mindfulness Scale

We estimated a linear mixed model that included session (pre and post) and condition (active and sham) as fixed effects, their interaction, and a random intercept for subjects, for the Toronto Mindfulness Scale score. The formula for the model is Toronto Mindfulness Scale Score \sim Session * Condition + (1 | Subject) (see Table 4). There was a significant main effect of session (p = 0.0001), with the post session having higher values than the pre session. However, the main effect of condition (p = 0.552) and the interaction between session and condition (p = 0.173) were not significant.

Table 4

Effects Estimates for the Linear Mixed Model for Toronto Mindfulness Scale

<table>
<thead>
<tr>
<th>Fixed Effect</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>df</th>
<th>t value</th>
<th>p-value</th>
</tr>
</thead>
</table>

Paired Wilcoxon rank-sum tests were performed to assess within-session differences for each condition and subscales ("curiosity" and "decentering") of the Toronto Mindfulness Scale (see Table 5 and Figure 5). Significant differences were found within the active condition between pre and post sessions for the total mindfulness score \( (t(14) = -4.51, p = 0.0004) \), the "curiosity" subscale \( (t(14) = -3.98, p = 0.001) \), and the "decentering" subscale \( (t(14) = -3.24, p = 0.006) \). Increases in the "curiosity" subscale suggest that participants became more open to novelty and more interested in their internal experiences, with less judgment towards them.

Increases in the "decentering" subscale reflect an improved ability to be detached towards one’s thoughts and feelings, avoiding identifying with them or perceiving them as accurate reflections of reality (Lau et al., 2006).

In the sham condition, significant differences were observed for the total mindfulness score \( (t(14) = -2.47, p = 0.027) \) and the "decentering" subscale \( (t(14) = -2.87, p = 0.012) \) but not the "curiosity" subscale \( (t(14) = -1.59, p = 0.134) \). The linear mixed-effects model did not show significant main effects or interactions overall, but the paired Wilcoxon rank-sum tests consistently indicated significant differences within the TFUS group and inconsistently or of smaller magnitude within the sham group.

**Table 5**

*Paired Wilcoxon rank-sum tests for Toronto Mindfulness Scale*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Comparison</th>
<th>t value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject (Intercept)</td>
<td>69.15</td>
<td>8.316</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>31.79</td>
<td>5.638</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Active  Pre vs Post (Total)  -4.509  14  0.0004***
Sham  Pre vs Post (Total)  -2.472  14  0.027*
Active  Pre vs Post (Curiosity)  -3.979  14  0.001**
Sham  Pre vs Post (Curiosity)  -1.590  14  0.134
Active  Pre vs Post (Decentering)  -3.242  14  0.006**
Sham  Pre vs Post (Decentering)  -2.870  14  0.012*

Note: *** p<0.0001, ** p<0.01, * p<0.05

Figure 5

Effects of TFUS on Toronto Mindfulness Scale
Figure 5. Boxplots depicting changes measured by the Toronto Mindfulness Scale. A. Changes in total mindfulness score between pre and post for each active and sham condition. B. Changes in the “Decentering” subscale. C. Changes in the “Curiosity” subscale. *** p<0.001, ** p<0.01, * p<0.05.

**Visual Analog Mood Scale**

We estimated a linear mixed model that included session and condition as fixed effects, their interaction, and a random intercept for subjects, for each score Global Affect and Global Vigor from the VAMS. The formula for the model is \([GA \text{ or } GV] \sim \text{Session} \times \text{Condition} + (1 | \text{Subject})\) (see Table 6). There was a significant decrease in GV from the baseline to the post-session in the active condition (Estimate = -9.500, SE = 3.686, \(t(28) = -2.578, p = 0.016\)), with no significant differences in GV observed between conditions at baseline (Estimate = -3.500, SE = 5.563, \(t(42.591) = -0.629, p = 0.533\)). The interaction between session and condition was not significant (Estimate = 2.500, SE = 5.212, \(t(28) = 0.480, p = 0.635\)). The random effects structure showed a subject-specific intercept variance of 130.2 (SD = 11.41) and a residual variance of 101.9 (SD = 10.09). For GA, there were no significant differences between sessions in the active condition (Estimate = 2.500, SE = 2.589, \(t(28) = 0.966, p = 0.342\)), with no significant differences in GA observed between conditions at baseline (Estimate = -4.167, SE = 4.420, \(t(39.116) = -0.943, p = 0.352\)). The interaction between session and condition was not significant (Estimate = 3.000, SE = 3.661, \(t(28) = 0.819, p = 0.419\)). The random effects structure showed a subject-specific intercept variance of 96.28 (SD = 9.812) and a residual variance of 50.27 (SD = 7.090).

**Table 6**

**Effects Estimates for the Linear Mixed Model for Visual Analog Mood Scale**

<table>
<thead>
<tr>
<th>Global Affect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>
Wilcoxon rank-sum tests a significant difference in GV between sessions in the active condition ($V = 75$, $p = 0.04238$), while no significant differences were observed in GA ($V = 32$, $p = 0.2053$). In the sham condition, there were no significant differences between sessions in GV ($V = 94$, $p = 0.05653$), or GA ($V = 26$, $p = 0.1015$). See Figure 6.

**Figure 6**

*Effects of TFUS on Visual Analog Mood Scale*
Figure 6. Boxplots depicting changes measured by the Visual Analog Mood Scale. A. Changes in Global Affect score between pre and post for each active and sham condition. B. Changes in the Global Vigor score between pre and post for each active and sham condition. A significant difference was found in the active condition. * p<0.05.

Self Scale

Wilcoxon rank-sum tests were performed for each item to compare conditions. Three significant differences (higher scores in active) were found in the items “I saw events from my past” (W = 142, p = 0.043), “My sense of time was distorted” (W = 142, p = 0.043), and “I lost all sense of ego” (W = 148.5, p = 0.020). See Table 7 and Figure 7.

Table 7

Wilcoxon rank-sum tests of Self Scale

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean Difference</th>
<th>Wilcoxon W</th>
<th>Wilcoxon p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I saw events from my past</td>
<td>2.81</td>
<td>142</td>
<td>0.043*</td>
</tr>
<tr>
<td>My sense of time was distorted</td>
<td>2.35</td>
<td>142</td>
<td>0.043*</td>
</tr>
<tr>
<td>I felt a profound inner peace</td>
<td>1.89</td>
<td>129.5</td>
<td>0.147</td>
</tr>
<tr>
<td>I lost all sense of ego</td>
<td>1.86</td>
<td>148.5</td>
<td>0.020*</td>
</tr>
<tr>
<td>I saw geometric patterns</td>
<td>1.66</td>
<td>120</td>
<td>0.308</td>
</tr>
<tr>
<td>It felt like I was floating</td>
<td>1.66</td>
<td>127</td>
<td>0.181</td>
</tr>
<tr>
<td>I felt like I was merging with my surroundings</td>
<td>1.64</td>
<td>130</td>
<td>0.140</td>
</tr>
<tr>
<td>The experience had dreamlike quality</td>
<td>1.61</td>
<td>130.5</td>
<td>0.134</td>
</tr>
<tr>
<td>Things looked strange</td>
<td>1.08</td>
<td>129.5</td>
<td>0.144</td>
</tr>
<tr>
<td>My thoughts wandered freely</td>
<td>1.07</td>
<td>127.5</td>
<td>0.174</td>
</tr>
<tr>
<td>I experienced a loss of separation from my environment</td>
<td>1.02</td>
<td>123</td>
<td>0.249</td>
</tr>
<tr>
<td>I felt unusual bodily sensations</td>
<td>0.97</td>
<td>118</td>
<td>0.356</td>
</tr>
<tr>
<td>I feared losing control of my mind</td>
<td>0.66</td>
<td>132</td>
<td>0.115</td>
</tr>
<tr>
<td>My thinking was muddled</td>
<td>0.57</td>
<td>115.5</td>
<td>0.419</td>
</tr>
<tr>
<td>I felt afraid</td>
<td>0.37</td>
<td>114</td>
<td>0.458</td>
</tr>
<tr>
<td>The experience had a spiritual or mystical quality</td>
<td>0.18</td>
<td>115.5</td>
<td>0.420</td>
</tr>
<tr>
<td>I felt suspicious and paranoid</td>
<td>-0.16</td>
<td>114.5</td>
<td>0.441</td>
</tr>
<tr>
<td>My sense of size and space was distorted</td>
<td>-0.20</td>
<td>105</td>
<td>0.747</td>
</tr>
<tr>
<td>I felt completely normal</td>
<td>-0.23</td>
<td>92.5</td>
<td>0.836</td>
</tr>
<tr>
<td>My imagination was extremely vivid</td>
<td>-0.29</td>
<td>84.5</td>
<td>0.564</td>
</tr>
</tbody>
</table>
Sounds influenced things I saw -0.48 94 0.890

*Note.* Items ordered by difference of mean values between active and sham conditions. *
p<0.05

**Figure 7**

*Effects of TFUS on Self Scale*

*Figure 7.* Individual item responses to the Self Scale. *p<0.05.*
**Post Experiment Questions**

No negative experiences or adverse events were reported. When asked to guess which condition they were in, 11/15 of participants in the active condition guessed “stimulation,” and 3/15 of those in the sham condition guessed “stimulation,” \(X^2 (3, N=30) = 8.571, p = 0.0356\). When asked if their mental state changed, 10/15 in the active condition said yes, and 5/15 in the sham condition said yes, \(X^2 (3, N=30) = 3.333, p = 0.3430\). When asked if there were any changes in their “inner talk- or thinking-space” descriptions of felt effects included “made less thoughts,” “better,” “drifted more,” “more calm,” “mind wandering less,” “more active lighter thoughts,” “less effort to think,” “less dark thoughts,” “more calm,” “somewhat more relaxed” in the active group, and “more fluid than usual,” “I was able to organize my thoughts for the day,” “talked more,” “smoother,” “calmed down” in the sham condition. Only 3/30 of participants in either condition said they heard sounds from the transducer, and 2 of those 3 reported that sound as the hum of the amplifier. The other 1 that reported a sound described it as a “buzzing” which may have referred to the actual sound of the transducer emitting at a subaudible PRF of 10.526 Hz.

**Discussion**

This study targeted the PCC with TFUS with the aim to reduce resting state functional connectivity in the DMN, predicting that this would result in phenomenological effects on mindfulness and self-referential processing. In an ROI-to-ROI test across the whole brain, the active TFUS group showed reductions in functional connectivity along the midline of the DMN. Additionally, we found that the active TFUS group showed multiple phenomenological changes, namely, an increase in state mindfulness as measured by the Toronto Mindfulness Scale, a reduction in Global Vigor as measured by the Visual Analog Mood Scale, and changes in items
related to the sense of ego, sense of time, and seeing memories from the past, as measured by the Self Scale.

We used linear mixed models to estimate a contrast between active and sham TFUS groups, and we did not find significant differences. Similarly, linear mixed models on the phenomenological measurements also did not yield significant effects. This is likely due, in part, to low statistical power for between-subject comparisons with the small sample size and to relatively low ultrasound power.

Our parameters yielded an ISPTA of 293 (mW/cm²) and an ISPPA of 5.58 (W/cm²) as measured in water. Other studies have used higher intensities on human subjects: Ai et al. (2018) had an Ispta of 6.102 W/cm² and an ISPPA of 16.95 W/cm²; Legon et al. (2018) had an ISPTA of 6.192 W/cm² and an ISPPA of 17.2 W/cm². Numerous animal studies have used even higher intensities to elicit their detected effects (Kim et al., 2014, 2022; Yoo et al., 2011; Yoon et al., 2019). Additionally, transmitting ultrasound through the posterior parietal portion of the skull is likely to significantly attenuate the amount of energy that gets transmitted (Mueller et al., 2017). Compared to Sanguinetti and colleagues (2020), who used the thin “temporal window” for transmission, we transmitted less energy to a deeper neural region. It is likely that higher intensities are needed for more consistent effects.

No significant effects were found in BOLD signal or pc-ASL analysis. This is most likely due to the low intensity of the ultrasound. It may be the case that the intensities used in this study represent the “floor” at which any effects are exhibited in brain activity. That such effects show up in BOLD functional connectivity but not simple BOLD signal suggests more of a tissue-level disruptive or suppressive effect than a straightforward inhibitory effect.

Given that we saw significant increases in mindfulness in the sham group, it is possible that the combination of the MRI scanner, the simulated brain stimulation, and the cues provided
by the Toronto Mindfulness Scale and VAMS scales caused subjects to become more present-centered and mindful in their attention. Despite that, the effect sizes in the Toronto Mindfulness Scale changes for the active group were consistently larger compared to the sham group, suggesting TFUS to the DMN may enhance state mindfulness. Future studies that employ increased TFUS power, better targeting methods (such as functional-based targeting), or that combine TFUS with mindfulness training may find a clearer link between DMN changes and state mindfulness.

This study was intended to be a proof-of-concept that it is possible to modulate DMN with focused TFUS. The intention was to make the parameters suppressive by using a low duty cycle. We targeted along the midline, aiming to hit both hemispheres of the PCC. DMN functional connectivity decreased, suggesting a disruption to the stability of the network, including left and right hemisphere locations along the midline decoupling from each other. Although we targeted ventral PCC, other areas like the dorsal PCC and precuneus may have also been in the path of the beam, which may have only added to the meditation-like effects that were observed (Garrison et al., 2015). Given that our tank measurements suggest the beam’s focus was shortened by the skull (see Figure 2 and Table 2), it is very possible that the precuneus was also affected.

Exploratory analysis of each individual time point revealed that the effects appeared more focused to the midline DMN in t₁ and then became more diffuse, spreading to more distal areas in t₂. This could indicate the path by which the offline effects dispersed throughout functional networks. In the sham condition, broad reductions were seen in somatomotor and dorsal attention networks in t₁, but these did not persist into t₂, and may be the result of a disengagement of attention with external environment and a switch to a more internal mode of processing. These exploratory results should be interpreted cautiously given the relaxed statistical threshold.
It is also worth noting that these effects persisted for at least 30 minutes (25 min + 6 min scan) after the application of TFUS. Sanguinetti and colleagues (2020) found offline increases in mood for at least 30 minutes following TFUS to the right dlPFC, Kim and colleagues (2023) found offline changes in resting state functional connectivity lasting more than an hour following thalamic stimulation, and Verhagen and colleagues (2019) found offline effects persisted for more than an hour in macaques. These long-lasting offline effects may be due to glutamate release via the opening of TRPA1 channels in astrocytes (Oh et al., 2019), opening up the possibility of long-term plasticity effects. The full temporal extent of these offline effects should be the subject of future research. However, in the post-experiment questions, participants, even those reporting subjective effects, reported they were back to baseline by the post-questioning (~1 hour post sonication).

We did not use masking or sham sound for the transducer because our PRF was in the subaudible range, and PRF is what is heard (Braun et al., 2020). Only one participant reported a “buzzing” that could conceivably be that sound. This, combined with the 11/15 of participants that correctly guessed they were in the active condition (compared to 3/15 for sham) suggests that the distinct subjective experiences played a role in their judgment.

The phenomenological effects of this treatment correspond with what one might predict would result in DMN disruption via the PCC (Brewer et al., 2013), and it demonstrates that TFUS has the potential to be used as an unprecedented neuromodulatory probe for deep brain sites. The brief subjective descriptions of “lighter” or “less” thoughts, “drifting,” and “less effort” correspond with Brewer et al.’s (Brewer et al., 2013) description of the PCC being involved in “getting caught up in” one’s thoughts. Given that we were able to reduce DMN functional connectivity in naïve undergraduate students, it would be fruitful to measure the same effects on experienced meditators. Individuals who have developed a richer interior clarity and
are more capable of manipulating at will their DMN activity might find an added benefit to meditation when TFUS is applied to their PCC, such as was found in PCC reduction neurofeedback studies with experienced meditators (Garrison et al., 2013; van Lutterveld et al., 2017).

We also found reductions in the sense of ego, though to a much lesser degree than what is seen in psychedelics (Lebedev et al., 2015). Our results were also not accompanied by sensory distortions or hallucinations, likely due to the fact the effects of TFUS were isolated to the DMN. This further demonstrates how the spatial specificity of TFUS can be used by researchers to generate causal models about functional brain networks.

These effects also suggest that TFUS shows promise as a therapeutic tool for disorders associated with DMN activity, such as depression and anxiety (Scalabrini et al., 2020). It may serve in other roles, too, given that it provides superior targeting abilities compared to other non-invasive brain stimulation techniques. The offline effects would allow for the TFUS to be embedded in a larger therapeutic intervention that corrects functional imbalances in the brain’s activity.

It should finally be noted that all of this was achieved with a relatively rudimentary targeting approach. Although individual MR-guided neuronavigation was used, the transducer was held by hand, the target was selected by anatomical structure alone, and no efforts were made to minimize skull aberrations. Future research can improve on all these circumstances with robot-controlled transducers, functional-based targeting, and skull aberration modeling. That we saw such significant effects with this approach is extremely promising for the future of TFUS.

Conclusion

This pilot study showed that TFUS targeted at the PCC can disrupt DMN activity and cause mindfulness-increasing subjective effects. Given these effects, TFUS may serve as a
therapeutic tool for treating network dysfunction. Future research should replicate these effects with a larger sample size, more precise targeting methods, and TFUS intensities matching previous human and animal studies. Future research may also investigate what ultrasound parameters, targeting, and modeling methods are optimal for neuromodulation.
CRediT authorship contribution statement

Brian Lord: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. Joseph L. Sanguinetti: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Writing – review & editing. Lisannette Ruiz: Investigation, Project administration. Vlad Miskovic: Conceptualization, Methodology, Supervision. Joel Segre: Conceptualization, Methodology, Supervision. Shinzen Young: Conceptualization, Methodology, Supervision. Maria E. Fini: Investigation, Formal Analysis, Software. John J.B. Allen: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

Conflict of interest statement

Author JLS is paid a salary and is a shareholder in Sanmai Technologies, PBC. Author LR is paid a salary by Sanmai Technologies, PBC. Authors JS and VM were paid a salary by X Moonshot Factory.

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Appendix C: Psychophysiological Effects of Transcranial Focused Ultrasound on Experienced Meditators

Psychophysiological Effects of Transcranial Focused Ultrasound on Experienced Meditators

Brian Lord, Jay Sanguinetti, Laura Beaman, Erica Cook, Maria Fini, John JB Allen

University of Arizona
Abstract
This study investigated the psychophysiological effects of transcranial focused ultrasound (tFUS) on experienced meditators, aiming to explore the interaction between tFUS neuromodulation and meditation. It was hypothesized that tFUS, when combined with meditation, might enhance the meditative state by modulating neural activity. A group of fifteen experienced meditators underwent sessions where tFUS was applied to the posterior cingulate cortex (PCC) using varying pulse repetition frequencies (PRFs) while they engaged in a body scan meditation. We measured changes in EEG power and phase components, nonlinear complexity, HRV, and subjective experiences of meditation. The results showed minimal changes across most parameters. No changes in frontal alpha power or HRV metrics like RMSSD were detected. EEG complexity metrics showed no significant changes. Subjective reports also indicated no differences between the three conditions. However, a significant reduction in inter-site phase clustering (ISPC) before and after sonication was observed in the alpha frequency range in the high pulse PRF condition, suggesting a potential area for further exploration. These findings are compromised by several limitations, including technical problems with the focused ultrasound transducer and participant discomfort.

Keywords: transcranial focused ultrasound, tFUS, EEG, HRV, neuromodulation, meditation, mindfulness
Introduction

Meditation and the beneficial effects of mindfulness training are an increasingly popular topic in science and medicine (Kabat-Zinn, 2019). The effects of long-term meditation practice include improvements in stress and well-being (Goyal et al., 2014), immune response and aging (Black & Slavich, 2016), and spiritual development and expanded consciousness (Vieten et al., 2018). The benefits of meditation practice often take many years of sustained practice to develop (Bauer et al., 2019; Hauswald et al., 2015), so interventions that can enhance the experience of mindful states or accelerate the acquisition of mindfulness skills would be welcomed. Efforts to enhance mindfulness with non-invasive brain stimulation (NIBS) have found modest but inconsistent results (Lord et al., submitted). A recent study used transcranial focused ultrasound (tFUS) on experienced Vipassana practitioners to enhance their meditative depth (Cain et al., 2024), building on previous work that targeted the posterior cingulate cortex (PCC) with tFUS to alter default mode network (DMN) and increase state mindfulness (Lord et al., submitted). This study aims to build on this previous research.

Transcranial Focused Ultrasound

TFUS is an emerging type of non-invasive brain stimulation (NIBS) that uses focused ultrasound waves to modulate functional brain activity with high spatial accuracy (Blackmore et al., 2019; Bowary & Greenberg, 2018; Kim et al., 2023). When used for neuromodulation, tissue is typically sonicated with low intensity sine waves. The five most fundamental parameters are the intensity, the sonication duration (SD), the fundamental or acoustic frequency (AF), the pulse repetition frequency (PRF), and the duty cycle (DC) (Zhang et al., 2021). The intensity of the beam, measured in power per area (W/cm²), determines the amount of energy transferred into the tissue, which is quantified by the thermal index (TI) and mechanical index (MI) (Aubry et al., 2023). TI estimates local temperature increase due to absorption of the ultrasound beam, and it is
typically most affected by the sonication duration (SD), the duration of the pulse itself (Aubry et al., 2023). Correspondingly, MI estimates the mechanical effects on the tissue, based on the peak negative pressure divided by the square root of the frequency of the ultrasound wave, and is typically most affected by the maximum amplitude of the beam (Aubry et al., 2023). The AF is the fundamental sine wave output of the transducer, the PRF is the rate at which the ultrasound beam is pulsed on and off, and the duty cycle (DC) is the percentage of time that the transducer is on during a given sonication period. Lower AF transmits more energy through the skull at the expense of spatial accuracy (Zhang et al., 2021).

The research on the effects of changing these parameters is uncertain and complicated, likely because there is a battery of mechanisms at play. A review by Dell’Italia and colleagues (2022) argued that DC was primarily responsible for whether tFUS exhibited inhibitory or excitatory effects. However, there is evidence of cell-type dependent (Wattiez et al., 2017) and state-dependent (Yang et al., 2021) effects, indicating that tFUS could induce bidirectional effects simultaneously, depending on the underlying cellular architecture. Yu and colleagues (2021) showed a cell-type response to differing PRFs when DC was kept constant. They suggested the neuron-type-specific effects are due to differing distributions of calcium ion channels and/or the shape and orientation of axons and dendrites, which affects their response to acoustic radiation force (ARF). Zadeh and colleagues (2024) targeted tFUS at the motor cortex using a 2% DC and saw inhibition of motor evoked potentials (MEP) lasting 30 min and 60 min at 10 Hz and 100 Hz PRF, respectively, but no effects at 1000 Hz PRF. They suggested these inhibitory effects were due to the excitation of GABAergic interneurons. Conversely, Zeng and colleagues (2024) used a 2, 5, and 10 Hz PRF on the motor cortex and observed excitation of MEPs, with 5 Hz having the largest effect, and higher DCs (10% and 15%) and longer sonication durations lengthening the duration of increased MEPs. They suggested that the theta-range PRFs
are ideal for inducing long-term plasticity (LTP) through GABAergic inhibition. This hypothesis is supported by Yaakub and colleagues (2023), who found reduced GABA levels at the posterior cingulate cortex (PCC) but not dorsal anterior cingulate cortex (dACC) when targeted by theta-burst TUS (5 Hz PRF and 10% DC), showing another instance of state- or cell type-dependent effects, accompanied by increases in functional connectivity with distant sites from both targets. On the contrary, Lord and colleagues (2024, submitted) found reductions in resting state functional connectivity when the PCC was targeted with a 10.526 Hz PRF and 5.26% DC. Sherman and colleagues (2024) found most motor cortex neurons were preferentially activated by only one of 10, 40, or 140 Hz PRFs, presumably due to the heterogeneity of mechanosensitive calcium channels within and across individual cells, independent of cell type. Given these results, there are clearly multiple mechanisms at play when tFUS is applied to in vivo brain regions, and tissue-level effects will be multivalent, exhibiting dose-, type-, state-, and location-dependent response. Proposed mechanisms include changing membrane conformation, neurotransmitter modulation, thermosensitivity, mechanosensitivity, flexoelectricity, acoustoelectricity, and microtubule resonance (Dell’Italia et al., 2022; Witte et al., 2007; Zhang et al., 2021).

In the domain of resting state EEG dynamics, research by Mueller and colleagues (2014) showed that tFUS (500 kHz AF, 1000 Hz PRF, 36% DC) altered the phase rate, a measure of how instantaneous phase is changing, in both beta and gamma frequency bands during sonication of the somatosensory cortex. Phase rate may be indicative of self-organization and signal transmission processes, so changes may indicate state transitions. The fact that they saw effects primarily in beta range may reflect a preferential effect on pyramidal cells driving excitatory post-synaptic potentials (EPSPs) in the cortex. These phase effects may indicate a mechanism by which tFUS can exert network-level effects. Given that phase relationships can govern functional
connectivity (Deco & Kringelbach, 2016), modulation of phase synchronization may modulate network dynamics.

**Meditation**

Lord and colleagues (2024) previously showed that tFUS-induced reductions in default mode network (DMN) functional connectivity were accompanied by mindfulness-related phenomenological effects. Both “curiosity” and “decentering” subscales of the Toronto Mindfulness Scale (Lau et al., 2006) showed increases, along with modulations of the sense of time and ego, suggesting that the tFUS exerted a disruptive effect, unseating normal DMN function in a manner loosely comparable to meditation and psychedelics. Earlier research on the PCC and meditation (J. Brewer et al., 2013; J. A. Brewer et al., 2011; J. A. Brewer & Garrison, 2014), suggests that suppression or disruption of the PCC causes the mind to get less “caught up” in its experience, thus enhancing equanimity. These effects occurred in participants with minimal meditation experience. A recent review of empirical trials to enhance mindfulness using non-invasive brain stimulation (Lord et al. 2024, submitted) claims that the PCC-disruption approach is an ideal paradigm for enhancing mindfulness because it targets equanimity processes in the PCC rather than executive functioning in the frontal cortex.

Cain and colleagues (2024) applied a similar protocol (650 kHz AF, 100 Hz PRF, 5% DC) to experienced vipassana practitioners and showed that PCC sonication enhanced self-reported intensity of meditation while caudate sonication enhanced meditation depth, with maximal effects occurring roughly 20 minutes after sonication. Using experienced meditators to evaluate the subjective effects of neuromodulation is advantageous because they have increased introspective accuracy as a result of their practice (Fox et al., 2012). Moreover, skilled meditators are able to reduce activity in their PCC during meditation, likely due to enhanced connectivity with dIPFC, which exerts an inhibitory effect (J. A. Brewer et al., 2011; J. A.
Brewer & Garrison, 2014; Jang et al., 2011). This increased neural flexibility may mean that experienced meditators (>500 hours of practice) have the capacity for a magnified response to PCC sonication in comparison to novices.

**Neurophysiological effects of meditation**

Meditation facilitates sustained attention, reduced cognitive stress, and enhanced emotional regulation; a unique state of relaxed alertness characterizes the meditative state. Corollaries of these effects are reflected in neurophysiological activity. Meditation has been shown to modulate both the electrical oscillatory activity of the brain as detected by EEG (Lee et al., 2018; Lomas et al., 2015) and parasympathetic cardiac control (Telles et al., 2013).

EEG research on meditation shows there are distinct signatures of the meditative state. Generally, theta and alpha power are globally increased, reflecting a relaxed and aware mind state (Lagopoulos et al., 2009). More specifically, frontal channels show increases in alpha and theta in both focused attention and open monitoring meditation styles (Lee et al., 2018). These features reflect the increased calm, relaxation, and internal focus characteristic of meditation. This study had participants perform a body scan meditation, which includes elements of both FA and OM (Travis & Shear, 2010). Nonlinear complexity features of EEG are also altered by meditation. Earlier research showed that entropy reduces while fractal dimension increases in the EEG signals produced under meditation (Vyšata et al., 2014). Both reflect mechanistic features of how meditation changes functional brain activity. According to the authors, entropy reduction may reflect greater EEG synchronization, while fractal dimension increase may reflect increased self-organization of hierarchical oscillations.

Meditation’s effects on the ANS can be inferred through EKG recording of cardiac activity. Heart rate variability (HRV) tracks the variation in inter-beat intervals (IBI) of heartbeats and is an indicator of parasympathetic activity and vagal control (Kromenacker et al.,
A higher HRV indicates more flexibility and resilience in the system, while a lower HRV can reflect things like stress, fatigue, or the presence of a chronic health condition (Billman et al., 2015). One HRV metric, the root mean square of successive differences (RMSSD) specifically measures short-term variability in heart rate, reflecting predominantly parasympathetic influence, which would indicate greater relaxation and reduction in stress (Berntson et al., 2005).

**Experiment Overview**

Building on this past research, this study had three main objectives: 1) replicate and extend earlier research on the subjective effects of tFUS targeting the PCC to enhance mindfulness; 2) evaluate these effects in the domain of EEG and HRV; and 3) evaluate the effects of different pulse repetition frequencies. Experienced meditators were recruited so that they could enter a meditative state simultaneously with the sonication process. It was hypothesized that this combination would enhance their meditation more effectively than either alone. Based on earlier pilot testing, it was hypothesized that a higher PRF would subjectively improve meditation. In the domain of EEG effects, it was hypothesized that there would be increased frontal alpha and theta power in meditation, and that active tFUS would induce further increases due to its synergistic interaction with meditation. It was also hypothesized that the tFUS would induce changes in the phase component of the EEG signals – online phase rate would change, following Mueller et al. (2014), and the inter-site phase clustering (ISPC) of electrode pairs P3-P4, CP3-CP4, and F3-F4 would decrease, indicating a mechanism by which disruption of the PCC and DMN occurs. Finally, it was predicted that the sample entropy (SampEn) would decrease and the Higuchi fractal dimension (HFD) would increase as a result of both the meditation and its interaction with active tFUS.
Method

Participants

Fifteen experienced meditators were recruited, defined as more than 500 hours of self-reported meditation experience. For their self-reported demographics, fourteen were White and one was Asian, eleven were male and four were female, and their average age was 54.9±15.9 years. All participants were fluent English speakers.

Disqualification criteria were a history of mental illness, head injury, cardiac problems, migraines, or epilepsy, current pregnancy, abnormal or uncorrected eyesight, current drug or alcohol abuse, or current psychotropic medication use.

Experiment Overview

Each participant made three visits to the lab on nonconsecutive days. On each day, EEG was recorded and one of three different conditions of tFUS was administered. Each participant received all three conditions, in a randomized and balanced design. Each visit followed a four-part sequence (see figure 1): 1) Planning period: participants asked to plan the rest of their day. This was intended to act as an “anti-meditation” that would engage their DMN. 2) Pre-tFUS Meditation: Participants were instructed to perform a type of body scan meditation in which both pleasant and unpleasant body sensations are welcomed with gentle acceptance. 3) tFUS Stimulation: one of three possible conditions of tFUS was administered while the participant continued meditating. 4) post-tFUS Meditation: participants continued meditating. Each segment lasted five minutes. At the end, subjective measures were recorded to capture aspects of their experience.
Figure 1. Data were gathered before, during, and after tFUS application. TFUS parameters varied on each day in a randomized design.

Transcranial Focused Ultrasound

Neuronavigation

Participants were seated in a chair, meditating with their eyes closed. An MRI-guided stereotactic system (Visor2, ANT Neuro, the Netherlands) was used to guide tFUS targeting. Individual MRIs were not acquired, so an averaged structural MRI was used to estimate the anatomical location of the PCC. The transducer was held firmly against the subject’s head with gel applied to the scalp.

TFUS Parameters

The focused ultrasound was delivered by a custom 4-channel ring transducer (Sonic Concepts, Bothell, WA, USA) with an outer diameter of 64 mm that uses a sealed membrane filled with degassed water for coupling, which is then housed inside a custom 3D-printed casing. The transducer was driven by an acoustic amplifier (TPO-203, Sonic Concepts, Bothell, WA, USA), with the ultrasound beam having an acoustic frequency (AF) of 500 kHz. Two different parameter sets were applied on different days, to test the effects of different pulse repetition frequencies, while keeping all other aspects roughly equivalent. PRFs were calibrated to avoid
either condition having harmonics of the other. In the high PRF condition, \( \text{PRF} = 909.09 \text{ Hz} \), pulse repetition period (\( \text{PRP} \)) = 1.1 ms, pulse duration (\( \text{PD} \)) = 70 \( \mu \text{s} \) or 35 cycles, duty cycle = 6.36%; In the low PRF condition, \( \text{PRF} = 6.37 \text{ Hz} \), \( \text{PRP} = 156.9 \text{ ms} \), \( \text{PD} = 10 \text{ ms} \) or 5000 cycles, duty cycle = 6.37%. In the sham condition, the transducer was held against the scalp, but no energy was emitted. See table 1.

Table 1

**TFUS Parameters for each condition**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Acoustic Frequency (AF)</th>
<th>Pulse Repetition Frequency (PRF)</th>
<th>Pulse Repetition Period (PRP)</th>
<th>Pulse Duration (PD)</th>
<th>Duty Cycle (DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High PRF</td>
<td>500 kHz</td>
<td>909.09 Hz</td>
<td>1.1 ms</td>
<td>70 ( \mu \text{s} ) (35 cycles)</td>
<td>6.36%</td>
</tr>
<tr>
<td>Low PRF</td>
<td>500 kHz</td>
<td>6.37 Hz</td>
<td>156.9 ms</td>
<td>10 ms (5000 cycles)</td>
<td>6.37%</td>
</tr>
</tbody>
</table>

*Note.* Parameters were assigned on the TPO-203 using PRP and PD. PRF was calculated from these values.

**Acoustic Intensity Measurements**

Acoustic intensity was measured using a custom-built water tank setup. Data were recorded using a needle hydrophone (HNR-0500; Onda, Sunnyvale, CA, USA) with a geometric diameter of 2.5 mm. Pressure and intensity were calculated from the voltage recordings from the hydrophone. MI was calculated with a derated peak pressure using the attenuation coefficient of soft tissue (.3dB/cm) (Abbott, 1999). The measured output of this wave in degassed free water shows a peak negative pressure of 0.512 MPa. The output of the beam through a hydrated sample of cadaver parietal bone showed a peak negative pressure of 0.143 MPa (a 72% decrease). Full properties are presented in table 2.

Table 2

**TFUS Intensity Measurements**
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Free Water</th>
<th>Skull</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Negative Pressure (MPa)</td>
<td>0.512 MPa</td>
<td>0.143 MPa</td>
</tr>
<tr>
<td>Mechanical Index (MI)</td>
<td>0.72</td>
<td>0.20</td>
</tr>
<tr>
<td>Thermal Index (TI)</td>
<td>1.80</td>
<td>0.14</td>
</tr>
<tr>
<td>$I_{SPPA}$ (W/cm$^2$)</td>
<td>8.885 W/cm$^2$</td>
<td>0.69 W/cm$^2$</td>
</tr>
<tr>
<td>$I_{SPTA}$ (mW/cm$^2$)</td>
<td>442.72 mW/cm$^2$</td>
<td>34.71 mW/cm$^2$</td>
</tr>
</tbody>
</table>

*Note.* Measurements are equivalent between the two conditions. Only PRF was changed.

**TFUS Delivery**

TFUS was delivered in 5-second stimulus intervals followed by 10-second rest periods. The pattern of [5s ON, 10s OFF] was repeated 20 times consecutively, totaling a 5-minute duration. Conditions were randomized for each participant and delivered in a double-blind design. A research assistant would set the parameters on the amplifier and cover the screen so that the main experimenter and participant could not see the settings on the device. A computer controlled the activation of the amplifier to synchronize the timing of tFUS pulses with the headphones and EEG acquisition software.

**Bone Conduction Headphones**

Bone conduction headphones were worn by the participants in all three conditions. In the low PRF and sham conditions, the headphones played a noisy square wave tone at 909.09 Hz during 5 second intervals in which the tFUS was on, designed to mimic the sound of the tFUS in the high PRF condition in which the PRF is audible. In the high PRF condition, no sound was emitted by the headphones.

**Subjective Measures**

After the meditation, participants were asked to respond to a series of surveys about their experience. They completed the Metacognitive Processes of Decentering Scale (Hanley et al., 2020), the Toronto Mindfulness Scale (Lau et al., 2006), and a custom-made self-report survey in which they responded to single-item ratings on a 0-100 scale for the depth, concentration, clarity, and equanimity of their meditation. Finally, they were asked to speak into a microphone in
response to the question, “Can you describe your experience?” to capture an open-ended description of their subjective experience. One participant failed to answer a single question on the Toronto Mindfulness Scale. This item was “interpolated” by taking the average of other questions in the same category.

**Psychophysiological Measurements**

**EEG/EKG Acquisition**

EEG was recorded from 25 scalp locations (Fp1, Fp2, F7, F8, F3, F4, FC5, FC6, Fz, T3, C3, Cz, C4, T4, CP1, CP2, CP3, CP4, T5, P3, P4, T6, O1, Oz, O2) using a custom-made Electrocap International EEG cap with tin electrodes arranged according to the 10-20 placement system. While recording, Cz was used as the online reference, and the ground was located just anterior to Fz. Both vertical and horizontal electrooculogram potentials were measured to estimate eye movement, and bipolar EKG electrodes were placed over the left and right trapezius to estimate heart rate activity. Impedance was kept below 20 kΩ. The sample rate was 1000 Hz. The signal was amplified through a SynAmps2 amplifier and recorded on a computer running Neuroscan Curry 9 software. EEG and EKG recordings were categorized into four recording segments: Planning, Pre-tFUS Meditation, tFUS Sonication, Post-tFUS Meditation.

**EKG Processing**

For the EKG signals, a bandpass optimal finite impulse response (optFIR) filter (Cook III & Miller, 1992) at 10-40 Hz with a length of 3 seconds was applied. The resulting filtered data from each 5-minute segment were then converted into a .txt file format for use with QRSTool software (Allen et al., 2007). QRSTool facilitated the identification and marking of each heartbeat, followed by a visual analysis for artifact removal. This yielded inter-beat intervals (IBIs). The data were segmented into 30-second non-overlapping segments. For each segment, the square root of the average of the squared differences between IBIs was taken to
calculate the root mean square of successive differences (RMSSD) of the signal, a type of heart rate variability (HRV) metric. The average of these RMSSD values for each segment was then taken to estimate the RMSSD for the session.

**EEG Processing**

To preprocess the EEG recordings, they were first re-referenced to the average reference, allowing the Cz electrode signal to be returned to the full dataset. They were then high-pass filtered using an opt-FIR filter at 1 Hz with a filter length of 1 second. Data were downsampled to 250 Hz. Artifact subspace reconstruction was used to identify and remove non-stationary artifacts.

The first three subjects used one of the mastoids as the online reference and a sample rate of 500 Hz. They were re-referenced to Cz, the mastoids were removed due to the electrical noise from the headphones, and then they were average referenced like other subjects. All data were downsampled to 250 Hz, so the later subjects being recorded at 1000 Hz should have minimal difference.

**Frequency Band Power**

Power spectral density (PSD) of the EEG data at each electrode were estimated using Welch’s method, where signals are segmented into 3s windows with 50% overlap and windowed using a Hamming window to minimize spectral leakage. The Fast Fourier Transform (FFT) is then applied to each segment to extract its frequency domain. The periodograms of all segments are averaged together and log-transformed to estimate the final frequency domain for each given section of EEG data. To estimate the power in different frequency bands, the sum of the PSD values was taken across the frequency bins within each band: delta 1-4 Hz, theta 4-7 Hz, alpha 7-
13 Hz, beta 13-30 Hz, and gamma 30-50 Hz. Individual Alpha Frequency (IAF) was estimated using a method described by Grosselin and colleagues (Grosselin et al., 2018).

**Phase Rate**

To extract the phase rate during the stimulation period, data were first segmented into 10-second epochs, with the first 5 seconds capturing the ON phase and the second 5 seconds capturing the OFF phase of the tFUS sequence. A half-second linear taper was applied to each side to reduce edge effects. Then the data were filtered with an optFIR filter in each canonical frequency band. The angle of the Hilbert transform was calculated to extract instantaneous phase, and then the derivative of the unwrapped signal was taken to compute the phase rate, which is given in radians. Dividing by $2\pi$ yields in Hz the speed at which the phase is changing. The mean of the absolute value was taken to estimate the average phase rate for each epoch. A higher value would indicate greater variability in the phase of a given frequency band, suggesting more dynamic neural activity, potentially in response to the tFUS neuromodulation.

**Inter-Site Phase Clustering**

Inter-site phase clustering (ISPC) is a type of phase-based functional connectivity metric that compares the relative phase differences between two channel sites. EEG signals were first convolved with a series of complex Morlet wavelets by multiplying the FFT-derived frequency domain with the wavelet spectrum. A family of linearly spaced frequencies between 1 and 50 Hz were used, and the number of cycles increased logarithmically from 4 to 8. The inverse FFT was then taken so that the angle of the complex signal could be extracted to determine the phase. ISPC for each frequency was then calculated as:

$$\text{ISPC}_{ab} = \frac{1}{N} \sum_{k=1}^{N} e^{i(\theta_{a,k} - \theta_{b,k})}$$  \hspace{1cm} (1)
Where $k$ is a single given epoch out of $N$ total epochs, and $\theta_{a,k}$ and $\theta_{b,k}$ are the phase angles at epoch $k$ in electrodes $a$ and $b$. ISPC was calculated for electrode pairs P3-P4, CP3-CP4 – electrode pairs surrounding the location of the tFUS transducer – and F3-F4.

**Complexity**

Nonlinear aspects of EEG signals can be used to quantify the chaotic dynamics of brain activity, potentially indicating emotional states, consciousness states, or information processing amount (jiayi et al., 2007; Kesić & Spasić, 2016; Vega & Noel, 2015; Vyšata et al., 2014). For each electrode, Higuchi Fractal Dimension (HFD) and sample entropy (SampEn) were calculated. HFD is used to quantify the fractal depth or self-similarity of a signal. Higher values indicate more self-similarity across a greater range of time scales. It is calculated by sampling data points at progressively larger intervals and measuring the slope of the log-log plot of these samples against the interval size (Higuchi, 1988; Kesić & Spasić, 2016). SampEn is a measure of the predictability of a signal. Higher values indicate increased unpredictability across a greater range of time scales. It is calculated by taking the negative natural logarithm of the probability that two similar short sequences of a given length remain similar for 1 more sample across a full time series (Delgado-Bonal & Marshak, 2019; Richman & Moorman, 2000). They were both calculated using previously written Matlab scripts (Lord, 2023b, 2023a).

**Results**

**Scales**

*Toronto Mindfulness Scale (TMS)*

The raw TMS scores are presented in figure 2. Z-transformed data were used for statistical analysis. There were no apparent differences between the three tFUS conditions. A linear mixed model, specified by “Toronto Mindfulness Scale Score ~ Condition + (1|Subject) + (1|Day)”, was estimated to assess the significance of the tFUS condition as a fixed effect.
Random effects were defined as participant number to account for individual variability and the day number to account for potential order effects. To isolate the effects of the tFUS condition, a “null” model was estimated containing only the random effects of participant number and day number. A permutation test was performed comparing this null model to the full model, and it indicated no significant fixed effect of tFUS condition. Similar null results were found for the Curiosity and Decentering subfactors of the Toronto Mindfulness Scale.

**Figure 2.**

*Total Toronto Mindfulness Scores by Condition*
Figure 2. Average scores for all participants are represented by thick black line with standard error for each condition. Individual scores for each participant are represented by dashed lines and points.

**Metacognitive Processes of Decentering Scale**

The raw MPoD scores are presented in figure 3. Z-transformed data were used for statistical analysis. There were no apparent differences between the three tFUS conditions. A linear mixed model, specified by “MPoD Score ~ Condition + (1|Subject) + (1|Day)”, was estimated to assess the significance of the tFUS condition as a fixed effect. Random effects were defined as participant number to account for individual variability and the day number to account for potential order effects. To isolate the effects of the tFUS condition, a “null” model was estimated containing only the random effects of participant number and day number. A permutation test was performed comparing this null model to the full model, and it indicated no significant fixed effect of tFUS condition.
Figure 3. Average scores for all participants are represented by thick black line with standard error for each condition. Individual scores for each participant are represented by dashed lines and points.

Self-Report Scale

Similar analyses were performed on the proprietary self-report scale. All items were averaged together to get an overall score. Z-transformed data were used for statistical analysis.
Results are presented in figure 4. There were no apparent differences between the three tFUS conditions. A linear mixed model, specified by “Self-Report Score ~ Condition + (1|Subject) + (1|Day)”, was estimated to assess the significance of the tFUS condition as a fixed effect.

Random effects were defined as participant number to account for individual variability and the day number to account for potential order effects. To isolate the effects of the tFUS condition, a “null” model was estimated containing only the random effects of participant number and day number. A permutation test was performed comparing this null model to the full model, and it indicated no significant fixed effect of tFUS condition. Similar analysis was performed on each individual item, and no significant effects were found.
Figure 4. Scores from individual self-report questions (intensity, clarity, concentration, equanimity, and depth) were averaged together. Average scores for all participants are represented by thick black line with standard error for each condition. Individual scores for each participant are represented by dashed lines and points.

HRV

RMSSD was estimated for each segment. Z-transformed data were used for all statistical analysis. A paired t-test was performed comparing the planning period to the pre-stimulation...
meditation period across all conditions to see if meditation influenced RMSSD. No significant effect was found, suggesting that participants struggled to differentiate their behavior between planning and meditation segments. See figure 5 for raw data. A linear mixed model, specified by “RMSSD ~ Condition*Segment + (1|Subject) + (1|Day)”, was estimated for the RMSSD based on the interaction of Condition and Segment, with random effects for individual subjects and different days. To isolate the effects of the tFUS condition, a “null” model was estimated containing only the random effects of participant number and day number. A permutation test was performed comparing this null model to the full model, and it indicated no significant fixed effect of tFUS condition.
Figure 5. RMSSD by Condition and Segment

RMSSD by Condition and Segment

*Figure 5.* RMSSD were calculated for each segment. Average scores for each condition are represented by thick lines with standard error for each segment. Individual scores for each participant are represented by dashed lines and points.

**EEG**

**Frequency Band Power**

Frontal alpha power was estimated by averaging together the values from all frontal channels (Fp1, Fp2, F7, F8, F3, F4, and Fz). A linear mixed model was estimated for the frontal
alpha power based on the interaction of Condition and Segment, with random effects for individual subjects and different days, yielding the equation (Frontal Alpha Power ~ Condition*Segment + (1|Subject) + (1|Day)). No significant effects were found. Results are visualized in figure 6. No significant difference was found between the planning segment and the meditation segments, suggesting that participants did not adequately differentiate between the two tasks. The same analysis was performed for the theta band, yielding no significant differences (see figure 7).

Figure 6.

_Frontal Alpha Power by Condition and Segment_
Figure 6. Frontal alpha power was calculated for each segment. Average scores for each condition are represented by thick lines with standard error for each segment. Individual scores for each participant are represented by dashed lines and points.

Figure 7.

Frontal Theta Power by Condition and Segment
Figure 7. Frontal theta power was calculated for each segment. Average scores for each condition are represented by thick lines with standard error for each segment. Individual scores for each participant are represented by dashed lines and points.

**Phase Components**

Phase rate was estimated for each canonical frequency band during the stimulation period, corresponding to when the tFUS was ON, and when it was OFF, replicating the approach of Mueller and colleagues (2014). These estimates indicated the instantaneous response of the phase angle to the tFUS neuromodulation. An effect in this domain might signify an underlying neural mechanism. For each frequency band and condition, a permutation test was performed comparing the phase rate of ON and OFF periods. No significant effects were found.

ISPC was estimated for electrode pairs P3-P4, CP3-CP4, and F3-F4 across the frequency spectrum for the pre and post segments in each condition. Paired t-tests were performed at each frequency bin to test for differences. In the high PRF condition, a significant difference appeared at 9, 10, and 11 Hz in the P3-P4 pair. See figure 8. The average effect size of this difference was \( d=0.5873 \), where the post-tFUS segment showed lower phase synchronization than the pre-tFUS segment. It should be noted that this was performed with an averaged signal reference montage. The analysis was repeated with surface Laplacian-transformed signals, and no differences were found.

**Figure 8.**

*Frequency spectra of ISPC between P3-P4 channels before and after tFUS*
Figure 8. Inter-site phase clustering (ISPC) was calculated between P3-P4 channels across the frequency spectrum in each condition. A paired t-test detected significant differences ($p<0.05$) in the alpha frequency range (9-11 Hz) of the high PRF condition, with an average effect size of $d=0.5873$. Alpha phase synchronization decreased between posterior electrodes P3-P4 following tFUS when a high PRF was used.
Complexity

SampEn and HFD values were averaged across all electrodes to get a single value for each segment. Z-transformed data were used for statistical analysis. For both metrics, the linear model “Complexity ~ Condition*Segment + (1|Subject) + (1|Day)” was used to estimate the fixed effects of the interaction between condition and segment, with random effects for individual subjects and days to account for individual differences and order effects, respectively. No significant effects were detected.
Figure 9. Higuchi Fractal Dimension (HFD) was calculated for each segment. The average across all electrodes was taken. Average scores for each condition are represented by thick lines with standard error for each segment. Individual scores for each participant are represented by dashed lines and points.
Figure 10. Sample Entropy by Condition and Segment

*Sample Entropy* was calculated for each segment. The average across all electrodes was taken. Average scores for each condition are represented by thick lines with
standard error for each segment. Individual scores for each participant are represented by dashed lines and points.

**Discussion**

This study evaluated the effects of tFUS sonication of the PCC in experienced meditators while they practiced meditation using two different PRFs and a sham stimulation condition. Minimal subjective effects were found. No changes in alpha or theta power were detected across any segments or conditions. No changes in complexity were detected either.

A difference was detected between pre- and post-tFUS conditions in the alpha frequency range of ISPC in the high PRF condition. This effect was found using an averaged signal reference montage, but not a CSD Laplacian-transformed montage. A surface Laplacian transform is known to eliminate volume conduction (Cohen, 2014). Given that the PCC is a generator of alpha bursts when the DMN is active (Rusiniak et al., 2018), the transform may have removed this deep source of alpha oscillation from the signals. While the effect is of moderate size (d=0.5873), and minimal other neurological effects were found, this result warrants further research.

**Limitations**

This study suffered from numerous limitations, including the tFUS equipment, the participants’ comfort level, and the duration of the sessions. To begin, structural MRIs for participants were not collected, limiting tFUS targeting accuracy. Without an individual structural MRI, such as used in (Badran et al., 2020), any individual structural differences that affect the location of the PCC will remain unknown.

The environment in which the experiment was performed was suboptimal for meditation. It was a laboratory setting with fluorescent lighting. Several participants reported that the chair
was uncomfortable to sit in for extended periods of time, impacting their ability to meditate. Many participants also reported that five minutes was not a long enough time to settle into a meditative state, and they felt unprepared for the tFUS. Similarly, only five minutes following the sonication was also reported as too short a time to have a deep experience. Finally, all the equipment – the bone conduction headphones, the EEG cap, and the tFUS – also promoted discomfort among participants, distracting many of them from their meditation.

It is unclear how consistently the ultrasound equipment was working throughout the duration of the experiment. Data were collected in two seasons – fall and spring 2023. The TPO box was serviced between these two periods, during which a damaged amplifier was repaired, making it difficult to ensure that transducer performance was consistent throughout the experiment. That said, there did not appear to be differences in the data between the two recording seasons. The general pattern of minimal effects was consistent.

Some of the EEG recordings showed visible electrical artifacts due to the pulsing of the tFUS transducer. These artifacts were able to be removed through ICA, but it is possible that those components included intrinsic neural activity, too, especially if an entrainment effect was occurring.

**Conclusions**

Minimal effects were detected in this study, likely due to several limitations in the design and execution of the experiment. Improved experimental design and technological performance can mediate these limitations.
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