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# Inhibition of midfrontal theta with transcranial ultrasound explains greater approach versus withdrawal behavior in humans

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ABSTRACT

Recent reviews highlighted low-intensity transcranial focused ultrasound (TUS) as a promising new tool for non-invasive neuromodulation in basic and applied sciences. Our preregistered double-blind within-subjects study ($N$ = 152) utilized TUS targeting the right prefrontal cortex, which, in earlier work, was found to positively enhance self-reported global mood, decrease negative states of self-reported emotional conflict (anxiety/worrying), and modulate related midfrontal functional magnetic resonance imaging activity in affect regulation brain networks. To further explore TUS effects on objective physiological and behavioral variables, we used a virtual T-maze task that has been established in prior studies to measure motivational conflicts regarding whether participants execute approach versus withdrawal behavior (with free-choice responses via continuous joystick movements) while allowing to record related electroencephalographic data such as midfrontal theta activity (MFT). MFT, a reliable marker of conflict representation on a neuronal level, was of particular interest to us since it has repeatedly been shown to explain related behavior, with relatively low MFT typically preceding approach-like risky behavior and relatively high MFT typically preceding withdrawal-like risk aversion. Our central hypothesis is that TUS decreases MFT in T-maze conflict situations and thereby increases approach and reduces withdrawal. Results indicate that TUS led to significant MFT decreases, which significantly explained increases in approach behavior and decreases in withdrawal behavior. This study expands TUS evidence on a physiological and behavioral level with a large sample size of human subjects, suggesting the promise of further research based on this distinct TUS-MFT-behavior link to influence conflict monitoring and its behavioral consequences. Ultimately, this can serve as a foundation for future clinical work to establish TUS interventions for emotional and motivational mental health.

## 1. Introduction

Transcranial ultrasound neuromodulation/stimulation (TUS) delivers low-intensity ultrasound non-invasively through the skull to alter neural activity [1–4]. It has been defined as a continuous or in most cases pulsed and focused ultrasonic wave, able to safely produce reversible neuromodulatory changes with the potential of side effects reported as similar to other forms of non-invasive brain stimulation [5]. Thus it is clearly separable from high-intensity transcranial focused ultrasound, which is usually a continuous wave used for rapid heating of targeted tissue for ablation using a different ultrasound parameter space [2]. TUS offers several benefits, such as high precision regarding energy dosage as well as target selection (including deeper brain structures) [6–8] and initiatives such as the International Transcranial Ultrasonic Stimulation Safety and Standards consortium (ITRUSST; https://itrusst.github.io/) are currently working on detailed guidelines to promote safe and valid TUS use to maximize its potential benefit for research and practical applications. In particular, TUS has been highlighted as promising with regard to potential clinical applications such as emotional and motivational affective mental health [9–11]. Our study focuses on how TUS targeting the right prefrontal cortex (PFC) can change human emotional and motivational affective states, cortical

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activity, and most importantly, for the first time shows consequences on a behavioral level.[2]

The TUS field has produced substantial basic research findings such as successful neuromodulation of deep brain circuits in primates [12–19] and human primary motor, somatosensory, or visual cortex [20–25] as well as potential mechanisms on a micro-physiological molecular and cellular level (e.g. flexoelectricity and conformational changes regarding membrane capacitance, sonoporation, mechanosensitive channels, and membrane waves) [1–4]. Concerning relatively more translation-focused TUS for human affect neuromodulation, a pilot project has been reported with clinical imaging ultrasound and chronic pain patients [26]. Targeting right PFC via the right transtemporal window led to improvements in subjective global affect, operationalized with self-report visual analogue mood scales (VAMS). This could be successfully replicated in healthy as well as depressed participants with a specifically manufactured device prototype for transcranial focused ultrasound neuromodulation [27,28], including additional evidence for TUS reducing self-reported anxiety and worry [27]. A detailed overview of these studies on human affect leading up our study is provided in Table 1. While subjective mood effects were replicated [26–28], an examination on an objective level, for example by including physiological and behavioral measurements, has remained a current challenge. A first analysis on a macro-physiological level was provided with functional magnetic resonance imaging (fMRI) from nine healthy participants, indicating that TUS targeting the right PFC could inhibit connectivity in regions identified as central for affect regulation and related conflict monitoring in earlier work [28]. For instance, evidence-based theoretical frameworks have highlighted the left prefrontal cortex as a center for approach motivation, while the right PFC has been assigned a key role for negative emotions, withdrawal motivation, and feelings of conflict [31–36]. The idea of TUS-induced inhibition in areas relevant for executing heightened affect regulation and related conflict monitoring as an underlying mechanism is supported by TUS modelling approaches. Given the applied parameters (Table 1), modelling would predict inhibition on a neuronal level [37,38].

As the central element of our study, objective behavioral measurements were included in addition to electroencephalography (EEG) for further insight on a macro-physiological level. These objective measurements were examined in a previously established experimental virtual T-maze task paradigm [39–42] that was developed to examine motivational conflict between approach versus withdrawal behavior (originating from Jeffrey A. Gray's Reinforcement Sensitivity Theory [43,44]). The T-maze paradigm enabled the collection of behavioral data in varying events that could induce motivational conflict between approach versus withdrawal behavior to a varying degree, while simultaneously measuring EEG without movement artefacts. This allowed the possibility of explaining choices in varying degrees of motivational conflicts between approach versus withdrawal behavior (as free-choice responses performed via continuous joystick movements) from preceding EEG. As we were interested in right PFC TUS effects in conflict-inducing T-maze events, we chose conflict-related midfrontal theta (MFT) as our EEG marker. MFT has been reliably identified as an indicator for conflict representation on a neuronal level, for example when experiencing anxiety in conflict situations [45–49]. This signal in the 4–8 Hz range originates from a region that was shown to be affected by right PFC TUS, namely the midcingulate cortex, which is in line with earlier findings on the interaction between lateral and medial prefrontal systems [28,50,51]. MFT could successfully explain specific types of related behavior in various experimental settings [52–58]. In particular,

[2] We focus on the term TUS as it has been recommended to us as the most concise and consistent term in the literature. The technique that we use in our study has also been described in terms like UNMOD (ultrasonic neuromodulation), FUN (focused ultrasound neuromodulation), tFUS (transcranial focused ultrasound), or LIFU (low-intensity focused ultrasound).

**Table 1**
Overview of right prefrontal cortex (PFC) transcranial ultrasound neuromodulation (TUS) studies regarding human emotional and motivational affective states.

| | **Sanguinetti et al. (2020) [28] experiment 1** | **Sanguinetti et al. (2020) [28] experiment 2** | **Reznik et al. (2020) [27]** | **Current study** |
|---|---|---|---|---|
| **Summary of general information and findings** | | | | |
| *Sample* | 48 healthy students | 9 healthy students | 24 depressed students | 152 healthy students |
| *Experimental focus* | Self-reflection post-TUS starting at ca. 10 min, ending at ca. 30 min | Self-reflection post-TUS starting at ca. 10 min, ending at ca. 30 min | Self-reflection post-TUS starting at ca. 10 min, ending at ca. 30 min | Virtual T-maze post-TUS starting at ca. 50 min, ending at ca. 100 min |
| *TUS-induced effects* | Self-report global affect ↑ | Self-report global affect ↑ | Self-report global affect ↑ | MFT ↓ |
| | | Self-report global vigor ↑ | Self-report global vigor ↓ | TUS-induced inhibition of MFT could explain … |
| | | fMRI connectivity in resting state networks related to emotion and mood regulation ↓ | Self-report anxiety ↓ Self-report worrying ↓ | … approach behavior ↑ … withdrawal behavior ↓ |
| **TUS specifications** | | | | |
| *f* | 0.500 MHz | 0.500 MHz | 0.500 MHz | 0.500 MHz |
| *PNP* | 1.27 MPa | 1.26 MPa | 0.65 MPa | 1.09 MPa |
| $I_{SPPA}$ | 54 W/cm$^2$ | 54 W/cm$^2$ | 14 W/cm$^2$ | 40 W/cm$^2$ |
| $I_{SPTA}$ | 130 mW/cm$^2$ | 272 mW/cm$^2$ | 71 mW/cm$^2$ | 199 mW/cm$^2$ |
| *PL* | 65 μs | 125 μs | 65 μs | 125 μs |
| *PRF* | 40 Hz | 40 Hz | 40 Hz | 40 Hz |
| *DC* | 0.26% | 0.50% | 0.26% | 0.50% |
| *TT* | 30 s | 120 s | 30 s | 120 s |
| *MI* | 1.79 | 1.79 | 0.92 | 1.54 |
| *TIC* | 1.08 | 2.27 | 0.59 | 1.66 |

*Notes.* All studies used the exact same specifically manufactured device prototype (https://thync.com/, product name: Neurotrek U+, including gel pads for optimal transducer-skull-connection provided by https://siliconesolutions.com/, product name: SS-6060). The device delivered TUS via a single element transducer, focused at 30 mm via a two-part lens. This setup was applied via electrode F8 to target the right PFC, for full details on the related acoustic beam modelling see Fig. 3. The table measurements were conducted with a calibrated hydrophone on a three-axis stage positioning system in a water tank with degassed water at the center of the emitted ultrasound beam (Onda, HN-500, https://www.ondacorp.com/). Since the hydrophone setups differed slightly between the different study measurements, the ultrasound output measurements differ slightly as well, even though the same specifications were used. This could be caused by differences in the lag from water degassing to the actual testing, but since the device has been used on multiple occasions, we can't rule out transducer degradation [29]. On a further note, as Reznik et al. [27] were the first study that used TUS to target the right PFC in a clinically affected sample, a specific option of the TUS device was utilized to reduce the overall device output. The device's "power" setting was set to ca. 50%, which enabled setting the overall output of the device to only ca. 50% of its usual output. All parameters fall within the safety limits of transcranial ultrasonic energy delivery in humans, as recommended by the International Transcranial Ultrasonic Stimulation Safety and Standards (ITRUSST; https://itrusst.github.io/documentation/safety.html and personal communication), based on guidelines from the Food and Drug Administration (FDA; https://www.fda.gov/media/71100/download), the British Medical Ultrasound Society (BMUS; https://www.bmus.org/policies-statements-guidelines/safety-statements/), and the American Institute of Ultrasound in Medicine (AIUM; https://www.aium.org/officialStatements/65). In particular, it has to be considered that the intensity of our applied ultrasound beam is strongly reduced by the human skull, as also indicated in

Fig. 3. fMRI = functional magnetic resonance imaging. MFT = electroencephalographic midfrontal theta. *F* = ultrasound frequency. *PNP* = peak negative pressure. $I_{SPPA}$ = spatial-peak pulse-averaged intensity. $I_{SPTA}$ = spatial-peak temporal-averaged intensity. *PL* = pulse length. *PRF* = pulse repetition frequency. *DC* = duty cycle. *TT* = total time. *MI* = mechanical index. *TIC* = cranial thermal index. Reported parameters based on guideline recommendations [1, 30].

higher MFT has been linked to a higher tendency for withdrawal-like behavior, for example heightened anxiety experiences in anticipation of public speaking situational threat, or heightened "anxious gambling" with less risky decisions in a risk-based game [55,58]. In addition, anxiolytic drugs reduce conflict-specific MFT [54]. Since right PFC TUS has been shown to lead to global mood enhancement and reduced anxiety as well as worrying, we would expect that right PFC TUS effects should also be reflected in a reduction of conflict-related MFT activity. Moreover, this reduced conflict-related MFT activity should be related to less anxious, withdrawal-like behavior as well as more risky, approach-like behavior. As further expansion of prior right PFC TUS studies, we wanted to expand the sample size (previously 9–48) as well as the post-TUS time that was of interest for our effects (previously 10–40 min) [26–28]. Also, previously found mood effects have ranged from relatively specific, such as worry reduction [27], to comparatively unspecific, such as global affect enhancement [26–28]. To shed more light on specific subjective mood changes, we used scales that differed to a certain extent from prior studies [26–28], namely the Self-Assessment Manikin (SAM) and a different set of VAMS [59,60].

The works reviewed above suggest a model, whereby right PFC TUS reduces conflict-related MFT and conflict-related negative affect. By reducing conflict in this way, TUS may reduce the tendency for withdrawal behavior and increase the tendency for approach behavior. We thus predict that MFT decreases following TUS but not Sham should explain greater approach (versus withdrawal) in T-maze events where motivational conflicts between approach versus withdrawal behavior could potentially occur. Additionally, we included a substantially larger sample size than previous studies to increase power and provide a good test of replication. The following hypotheses were formulated.

**H1**. The mood of the participants will become more positive following right PFC TUS (versus Sham) over the course of the experimental session in general.

**H2**. Right PFC TUS (versus Sham) will induce a decrease of conflict-related MFT in the approach/withdrawal conflict events of the experimental virtual T-maze task paradigm.

**H3**. The MFT reduction induced by right PFC TUS (versus Sham) will explain greater approach (versus withdrawal) behavior during conflict events of the experimental virtual T-maze task paradigm.

This paper is based on an Open Science Framework (OSF) preregistration. Consequently, its most central elements have already been described in this preregistration (e.g., *H1* and *H2* as confirmatory hypotheses [61]), but we also included new elements based on our original ideas (e.g., *H3* as an exploratory hypothesis [61]). For details, see https://osf.io/9fqkz?view_only=edef266d57c14c3aa3395fc740c66dd8.

## 2. Results

### 2.1. Data analysis

***Preprocessing.*** Three relevant behaviors were classified: "approach into the T-maze", "(turn and) approach safety outside of the T-maze" and "withdrawal out of the T-maze". Fig. 2 illustrates which behaviors could achieve optimal outcomes for each event type. EEG was preprocessed according to a published automatized open source pipeline including current source density transformation for optimal scalp topography effect localization, see Table S2. Theta frequency (4–8 Hz) was extracted using Morlet wavelets during 250 ms – 350 ms after event cue appearance. MFT was extracted from EEG position FCz. Behavior classification was done using MATLAB R2015b and Neural Network Toolbox 8.4 (https://www.mathworks.com/products/matlab.html), a detailed guideline has been published [42]. A detailed guideline on the applied EEG preprocessing has been published as well and was implemented using MATLAB R2015b and EEGLAB [62,63].

***Statistics.*** To test *H1* ("The mood of the participants will become more positive following right PFC TUS (versus Sham) over the course of the experimental session in general."), we calculated 2 (TUS condition: TUS/Sham) x 3 (mood measurement times within each session: baseline/pre-task/post-task) within ANOVAs. The related statistical null hypothesis for this ANOVA would be "The means of our subjective mood scales after TUS application do not significantly differ from the means of our subjective mood scales after Sham application.". As we would also expect the means of TUS and Sham to be not significantly different at baseline (as this was measured prior to the TUS/Sham application) but then significantly different at pre-task and post-task (as these were measured post-TUS/Sham), we would expect a significant interaction in our ANOVA analysis to reject this statistical null hypothesis. Given a significant interaction was found, we would calculate the relevant post-hoc tests to investigate whether the more specific significant mean differences would be according to *H1* (meaning significantly more positive means for TUS compared to Sham at pre-task and/or post-task for "valence" (SAM), "happy" (VAMS), and "energetic" (VAMS), as well as significantly more negative means for TUS compared to Sham for "arousal" (SAM), "sad" (VAMS), "tired" (VAMS), "anxious" (VAMS), "tense" (VAMS), "angry" (VAMS), and "confused" (VAMS)).

To test *H2* ("Right PFC TUS (versus Sham) will induce a decrease of conflict-related MFT in the approach/withdrawal conflict events of the experimental virtual T-maze task paradigm."), a 2 (TUS condition: TUS/Sham) x 5 (T-maze event types: negative/negative-and-positive/ambiguous/positive-and-positive/positive) within ANOVA was calculated. The related statistical null hypothesis for this ANOVA would be "The MFT means after TUS application do not significantly differ from the MFT means after Sham application.". As we would expect the MFT means after TUS to be significantly decreased in comparison to the MFT means after Sham, we would either expect a significant main effect of the two-level factor "TUS condition" and/or a significant interaction in our ANOVA analysis to reject this statistical null hypothesis. Given a significant main effect of the two-level factor "TUS condition" and/or a significant interaction was found, we would calculate the relevant post-hoc tests to investigate whether direction of the significant mean differences would be according to *H2* (meaning significantly less positive MFT means for TUS compared to Sham). Given a main effect of the five-level factor "T-maze event types" was found, post-hoc tests were also calculated to investigate the more specific significant differences in MFT means between the different T-maze event types.

Given our statistical analysis related to *H2* detected relevant significant effects, linear regressions were computed to test *H3* ("The MFT reduction induced by right PFC TUS (versus Sham) will explain greater approach (versus withdrawal) behavior during conflict events of the experimental virtual T-maze task paradigm."). The related statistical null hypotheses for these regressions would be "The MFT differences of TUS-minus-Sham as the independent regression variable are not negatively related with the TUS-minus-Sham differences in withdrawal behavior frequency as the dependent regression variable." and "The MFT differences of TUS-minus-Sham as the independent regression variable are not positively related with the TUS-minus-Sham differences in approach behavior frequency as the dependent regression variable.". As we would expect the MFT differences of TUS-minus-Sham as an independent explanatory regression variable to be significantly negatively related to the TUS-minus-Sham differences in withdrawal frequency as well as significantly positively related to approach behavior frequency, significant regression coefficients in the according directions would lead

to rejection of these statistical null hypotheses.

For all analyses, alpha-error-level was conventionally set to $p = 0.050$ and Bonferroni-Holm correction was implemented against multiple post-hoc tests alpha inflation. If sphericity could not be assumed in the ANOVA analyses based on Mauchly's Test of Sphericity, Greenhouse-Geisser correction (*GGc*) was applied. Cohen's *d* was reported as an effect size for post-hoc tests, $\eta_p^2$ for ANOVAs, and standardized regression coefficients (*Beta*) were reported for linear regression effect interpretations. Jamovi 2.2.2 (https://www.jamovi.org) and IBM SPSS Statistics 27 (https://www.ibm.com/analytics/de/de/technology/spss/) were used for descriptive as well as inferential statistical calculations.

### 2.2. Self-report mood scales before and after the virtual T-maze task

VAMS data were analyzed in 139 cases out of the $N = 152$ (13 missing due to software problems). No significant interactions were observed in any of the 2 (TUS condition) x 3 (mood measurement times within each session) within ANOVAs for SAM and VAMS. TUS-independent main effects of the mood measurement times were found for "valence", "happy", "sad", "energetic" and "tense", significantly decreasing within each session, as well as "tired", significantly increasing within each session. For a detailed description of these TUS-independent effects see Fig. S1.

### 2.3. Conflict-related midfrontal theta (MFT) in the virtual T-maze task and TUS-induced MFT-based explanation of approach versus withdrawal behavior

An overview of the right PFC TUS effects on conflict-related MFT in the virtual T-maze task and the TUS-induced MFT-inhibition-based linear regression explanation of approach versus withdrawal behavior is illustrated in Fig. 1.

In the 2 (TUS condition) x 5 (T-maze event types) within ANOVA, a significant TUS condition main effect ($F(1, 151) = 6.199$, $p = 0.014$, $\eta_p^2 = 0.039$) and a significant main effect of T-maze event type emerged ($F(4, 604) = 50.020$, $p < 0.001$, $\eta_p^2 = 0.249$, $GGc = 0.658$), while the interaction did not reach significance. This TUS condition main effect difference between TUS ($M = 12.77$, $SD = 0.81$) and Sham ($M = 12.89$, $SD = 0.77$) resulted in Cohen's $d = 0.20$ ($M_{differences} = 0.12$, $SD_{differences} = 0.593$, $p = 0.014$), see Fig. 1*A*.

Our analysis centered on the event types with behavioral variations, namely, "ambiguous", "negative-and-positive", and "negative". (No meaningful behavioral variation occurred in the "positive" and "positive-and-positive" event types, with "approach into the T-maze" dominating in >98.00% of the trials.) Post-hoc t-tests revealed that conflict-related MFT was highest for "ambiguous" ($M = 12.97$, $SD = 0.76$), significantly surpassing "negative-and positive" by $d = 0.50$ ($M_{differences} = 0.16$, $SD_{differences} = 0.32$, $p < 0.001$) and "negative" by $d = 1.04$ ($M_{differences} = 0.38$, $SD_{differences} = 0.37$, $p < 0.001$). The post-task manipulation check showed a similar pattern, where "ambiguous" was significantly rated as the most conflict-inducing event in subjective hindsight, details see Fig. S2.

Due to its significant differences regarding conflict-induction, "ambiguous" was selected as the focus of the linear regression analysis that used the TUS-induced MFT-inhibition to explain behavior. For the "ambiguous" event type, greater TUS-induced MFT inhibition explained greater "approach into the T-maze" ($Beta = 0.205$, $p = 0.011$) and less "withdrawal out of the T-maze" ($Beta = -0.211$, $p = 0.009$). The reported *Beta*-values were distinctly linked to the TUS-induced MFT inhibition, as observable in the scalp topographies in Fig. 1*B*.

Significant explanation of increased approach and decreased withdrawal by TUS-induced MFT inhibition was also found for "negative-and-positive" and "negative", details see Fig. S3*A* and Fig. S3*B*. For a short summary of all relevant regression results see Table S1.

## 3. Discussion

Right PFC TUS significantly decreased conflict-related MFT (*H2*), explaining greater approach and fewer withdrawal behaviors (*H3*) in an experimental virtual T-maze task paradigm. The TUS-related MFT decrease was present across all T-maze event types, involving various events that induced motivational conflict between approach and withdrawal behavior, and showed a distinct scalp topography. This further supports the function of midfrontal theta as an indicator for conflict representation on a neuronal level [45–49]. Moreover, it shows that TUS can alter neural conflict representation and therefore was predicted to alter conflict-related behavior. Consistently, TUS-induced MFT differences explained greater approach and less withdrawal for T-maze event types where motivational conflict between approach and withdrawal behavior was evident. We focused on the "ambiguous" event type, where positive and negative outcome options occurred with a 50/50% chance and which was experienced as the most conflict-inducing event type. Here, approach behavior was potentially more risky (allowing more positive outcomes, but possibly resulting in negative outcomes) and withdrawal behavior was potentially less risky (not allowing more positive outcomes, but safely preventing negative outcomes). A similarly distinct regression pattern was observable for the other event types that had the potential for motivational conflict between approach and withdrawal behavior ("negative-and-positive", "negative"), even in an exclusive avoidance context ("negative"). Thus, it may be argued that the MFT reduction leads to a decrease in behavioral aspects of anxiety-like inhibition and withdrawal, while promoting approach behavior. This supports numerous studies, where heightened MFT has served as a significant explanatory variable for decreases in risky, approach-like behavior and increases in anxious, withdrawal-like behavior [52–55,58]. Notably, the scalp topographies revealed that even though MFT inhibition is central for behavior explanation, further mechanisms might be involved depending on specific conflict situation characteristics. Taken together, right PFC TUS seems to increase approach and decrease withdrawal tendencies through influences on systems that generate MFT.

Contrary to predictions (*H1*), TUS did not result in subjective mood changes seen in earlier studies [26–28]. Significant changes were observed but were TUS-independent (increasing tiredness and decreasing energy, valence, happiness, sadness, and tenseness). The absence of TUS-induced mood effects may stem from two key differences. First, previous studies found mood effects peaking at around 30 min post-TUS, a time when participants in our study were undergoing EEG recording preparation. This might have offset such effects or missed them, due to lack of mood assessment at this time. Second, prior studies allowed considerable internal self-reflection, whereas our study demanded external focus on a T-maze task, which has been identified as a strong situational manipulation [41]. In this respect, the TUS-induced MFT inhibition and its behavioral consequences are especially remarkable since they manifested despite the task's situational manipulation power and the TUS-independent emotional effects. Our effects are also substantially longer lasting then those reported in the aforementioned TUS mood studies that target the right PFC [26–28], occurring in a time window that started at approximately 50 min post-TUS (with the beginning of our virtual T-maze task) and ended at approximately 100 min post-TUS (with the ending of our virtual T-maze task), see also experimental session structure in Table 2. This compliments findings from animal research, where transient and safe effects on behavior were identified as sometimes lasting between half an hour and a day [12–19]. Overall, we would argue that our TUS-induced objective effects are in line with prior studies in terms of revealing positive consequences of right PFC TUS on human emotional and motivational affective states, expanding the evidence for the impact of right PFC TUS on physiology and behavior. On another note, the absence of subjective TUS versus Sham effects and the presence of more subtle but distinct relatively long-lasting objective TUS versus Sham effects supports the validity of

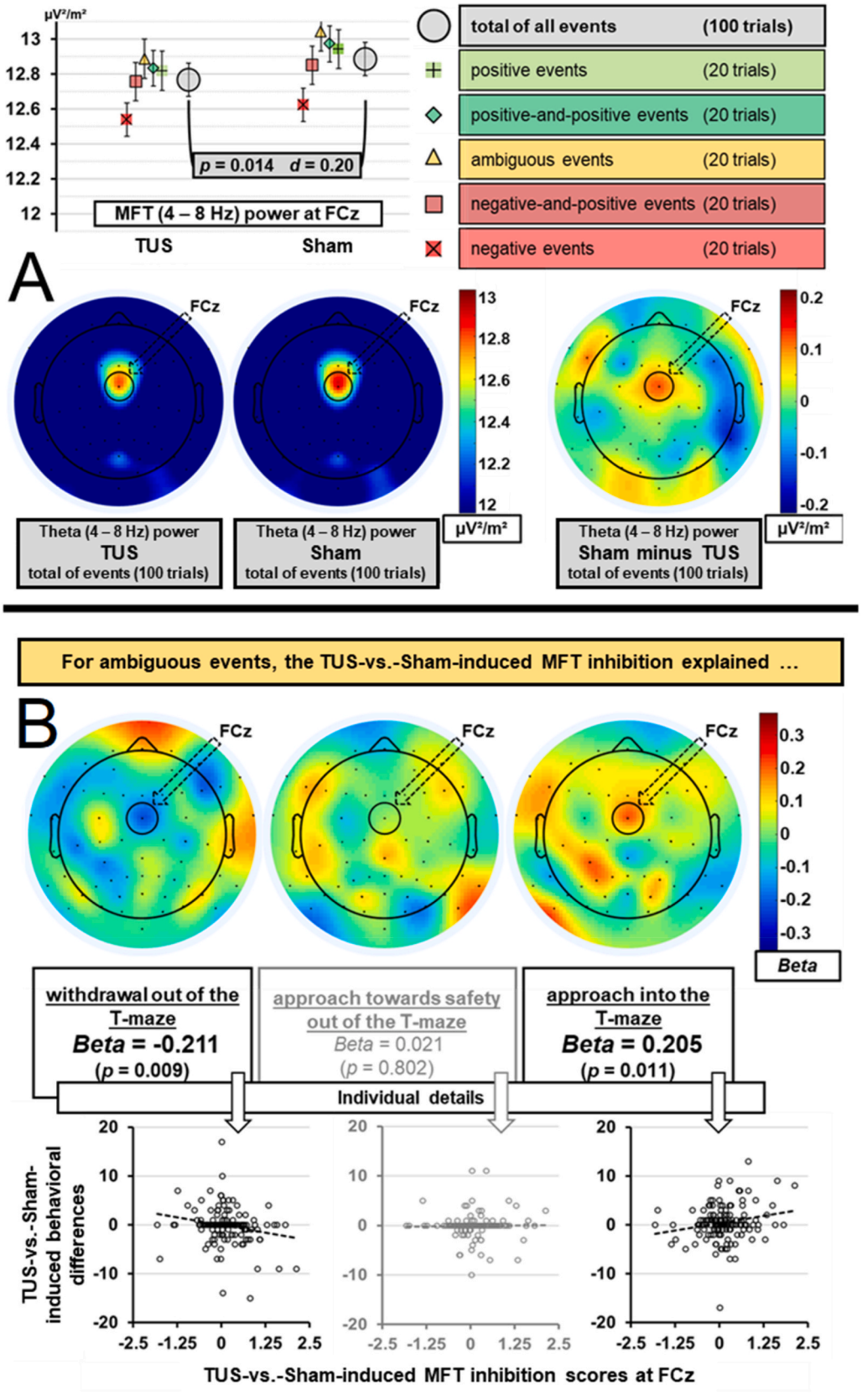


**Fig. 1.** Overview of the induced right prefrontal cortex low-intensity transcranial focused ultrasound (TUS) versus Sham differences in electroencephalographic (EEG) conflict-related midfrontal theta (MFT) and related behavior explanation. EEG was current source density transformed for optimal scalp topography effect localization. (A) TUS-induced MFT inhibition as measured at EEG position FCz. Error bars mark 95% confidence intervals of the mean of the TUS-versus-Sham-differences. TUS-induced significant MFT inhibition over the total of all T-maze events (Fig. 1*A* top), distinctly observable in EEG scalp topography (Fig. 1*A* bottom). (B) Explanation of the frequency differences of individual participants' behaviors for "ambiguous" event based on TUS-induced MFT inhibition. Scalp topographies plot the electrode specific standardized regression coefficients (*Beta*) and show distinct patterns (Fig. 1*B* top). Scatter plots show individual details (Fig. 1*B* bottom).

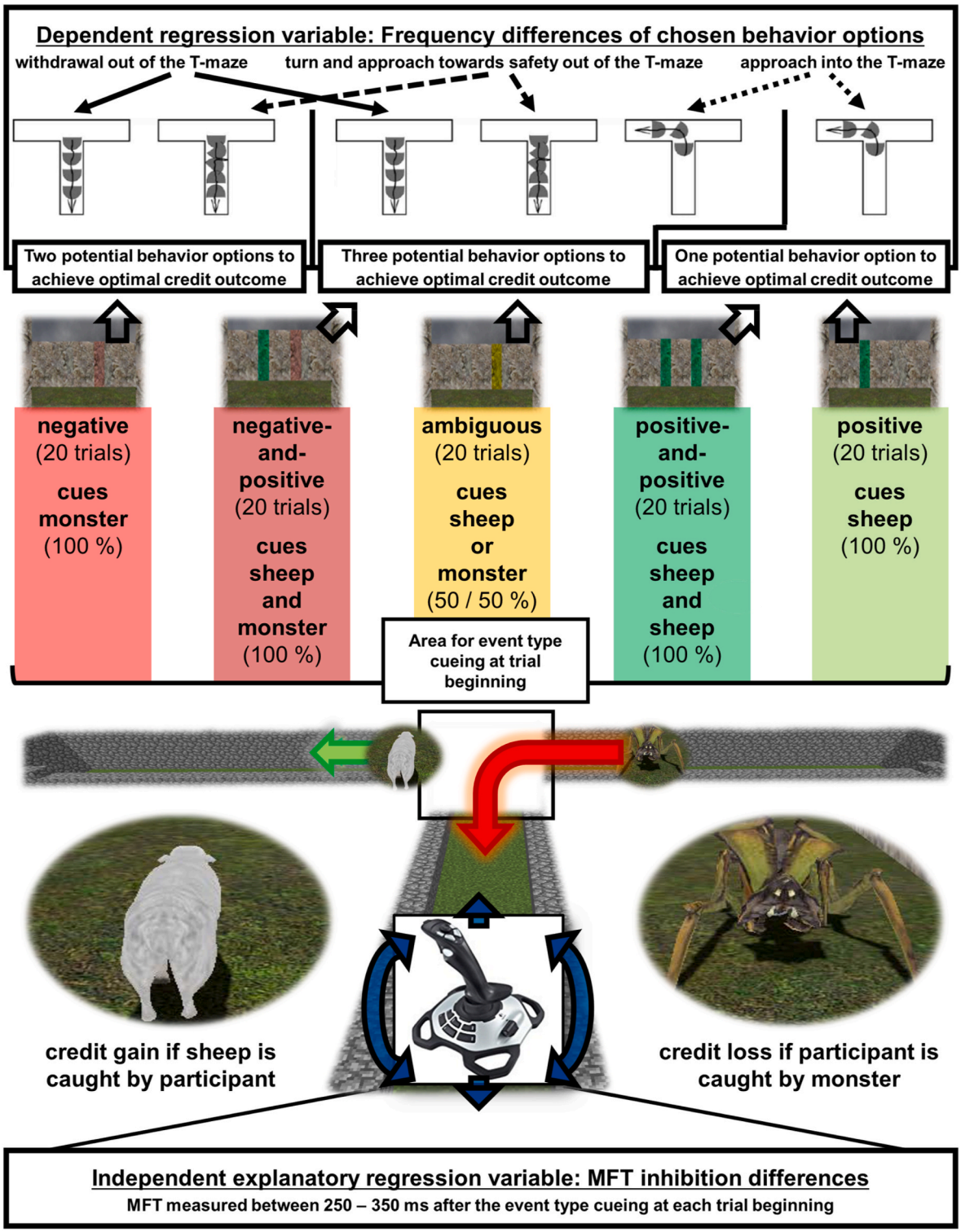


**Fig. 2.** Schematic of virtual T-maze task paradigm with five different event types, cued by specific event cues. The task of the participants was to collect as many virtual credits as possible. As the basis for the explanation of the behavior options in the different event types, conflict-related midfrontal theta (MFT) was measured in each event at electroencephalography position FCz. The MFT inhibition differences that were induced by right prefrontal cortex low-intensity transcranial focused ultrasound versus Sham, were used to explain the frequency differences of individual participants' chosen behavior options. For details, see "Methods" supplementary material including Movies S1 and S2, illustrating our paradigm in full motion.

our Sham control condition. If TUS and Sham had been clearly distinguishable, stronger effects would have been expected on a subjective and objective level due to demand characteristics. Furthermore, our findings are consistent with evidence from transcranial direct current stimulation and transcranial magnetic stimulation, where similar right PFC targeting results in similar effects on emotional and motivational affective states and has been implemented for therapeutic use [64,65].

TUS in humans is a relatively new field with many remaining challenges. We used a TUS device prototype that was specifically developed for ease-of-use and enabled us to collect a large sample size but did not

**Table 2**
Experimental session structure of our double-blind within-subjects study.

| Timeline | Experimental protocol steps | [measurement times of relevant data] |
|---|---|---|
| *0–10 min* | 1st step: Briefing (including time for open comments and questions) | [SAM & VAMS mood baseline measurement at ca. 5 min] |
| *10–15 min* | 2nd step: TUS or Sham (double-blind and randomly counterbalanced within subjects) | – |
| *15–65 min* | 3rd step: 64 channel EEG setup … (followed by three channel electrocardiography on collarbones and left costal arch and two channel electrodermal activity on left hand for separate analyses) … and 8 min resting EEG (for ensuring signal quality as well as participants' comfortability with the setup, e.g., seating position) | [SAM & VAMS mood pre-task measurement at ca. 65 min] |
| *65–115 min* | 4th step: Virtual T-maze task | [including a total of 100 trials with MFT and behavioral measurements] |
| *115–120 min* | 5th step: Debriefing (including time for open comments and questions) | [SAM & VAMS mood post-task measurement at ca. 115 min] |

*Notes.* All $N = 152$ participants completed two almost equally structured sessions that were separated by exactly seven days (unless participant rescheduling was required). The only difference between those two sessions was whether TUS or Sham was applied in the second step of the experimental protocol. To ensure that participants were familiar with our procedures, these two sessions were preceded by a familiarization session, which did not involve TUS or Sham and lasted ca. 15–30 min longer, since participants where not yet familiar with the paradigm. EEG = electroencephalography. TUS = transcranial ultrasound neuromodulation. MFT = midfrontal theta as measured at EEG position FCz. SAM = Self-Assessment Manikin. VAMS = visual analogue mood scales.

allow taking advantage of the high precision TUS spatial targeting and energy dosage potential. This would be expected to add variance to the TUS impact due to variations in targeting location based on individual participants' skull and brain structure. Future studies could utilize MRI-guided or even fMRI-guided precision targeting that could be combined with multi-transducer devices and provide more control over depth and focus of energy delivery [66–68]. This could shed further light on causal mechanisms and address whether small area targeting within the right PFC or more global targeting would be optimal for TUS effects. Regarding the underlying causal mechanisms of our observed effects as well as other aforementioned relatively long lasting transient and safe effects from animal research [12–19], a challenge for future work in noninvasive neuromodulation per se also lies in illuminating the relationship between immediately observable neuromodulation effects (*online* effects) and subsequent aftereffects (*offline* effects). To this end, a recent and detailed discussion of potential hurdles and solutions for inferring causality from noninvasive brain stimulation in cognitive neuroscience has provided suggestions for specifically tailored study designs [69]. In this context, we also want to highlight recent work on potential auditory confounds of TUS effects [70–73]. These phenomena have been predominantly described as a problem of online studies with immediately observable effects, but they might affect offline studies that investigate subsequent aftereffects of neuromodulation as well. As described earlier in the discussion section, we would argue that the absence of subjective TUS versus Sham effects and the presence of more subtle but distinct and relatively long-lasting objective TUS versus Sham effects supports the validity of our Sham control condition. Yet, we would also encourage future studies to include more varied control conditions, including auditory masking or ramped ultrasound onset, which our current TUS device did not allow for. In addition, exploring responder characteristics might be promising, especially regarding potential practical applications. TUS could ultimately be used as a complementary intervention, exemplarily before psychotherapy sessions for emotional and motivational mental health problems, as it has also been suggested by recently published work on TUS-induced modulation of learned helplessness via influencing midline theta by targeting the right PFC and right PFC TUS effects on MFT in the context of a control illusion task [74,75]. Knowledge about responder characteristics (e.g., consideration of individual skull characteristics [76]) would enable user-specific parameter optimization to prevent unintended effects and induce intended effects. As an important closing thought, we would like to point out that many definitions and operationalizations of conflict exist in various fields of neuroscience and psychology. Our specific virtual T-maze paradigm focuses on motivational conflict regarding decisions of choosing between approach versus withdrawal behavior as a free-choice task where the behavioral responses are executed via continuous joystick movements that can last up to 7000 ms. Manifold research on conflict experiences and related behavior has provided various other paradigms that could be utilized by future studies to clarify the effects of neuromodulation on different forms and operationalizations of conflict experiences and related behavior. For example, while a free-choice motivational conflict paradigm can illuminate potential differences in the frequencies of approach versus avoidance responses, a forced-choice motivational conflict paradigm can illuminate potential differences in the reaction times of approach versus avoidance responses [77]. A focus on potential differences in reaction time measures can provide more fine-grained examinations of motivational conflict experiences and could for example be implemented with a virtual T-maze paradigm that measures participant reactions with distinct button presses instead of continuously executed joystick movements, which would also allow to analyze event-related potential data if an EEG setup is applied [78]. For future experimental setups to examine the TUS-induced MFT-behavior link, more complex analysis methods such as mediation analysis or multilevel modelling could allow more complex conclusions. As more complex analysis methods might also require specific study design considerations (e.g., a between-subjects study design might be favorable over a within-subjects study design, or a specific paradigm might be favorable over another paradigm), this should be kept in mind from the very beginning of study planning [79–81].

## 4. Conclusions

This study revealed long lasting right PFC TUS effects on physiology and behavior with a large sample of human subjects. Our demonstrated TUS-MFT-behavior link to influence conflict monitoring and related increases in approach / decreases in withdrawal behavior merits further basic as well as applied research and can be a foundation for utilizing TUS for clinical interventions.

## 5. Methods

### 5.1. Participants

For participation in our study at the University of Würzburg, the following criteria had to be met (based on our experiences from earlier work with the virtual T-maze paradigm [39–42]): 18–35 years of age, right-handedness, absence of color blindness and neurological or psychiatric disorders. Informed consent was obtained from all participants, and all were compensated financially or via student course credits. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Psychological Institute of the University of Würzburg (approval number: GZEK 2017–18) [82]. Based on effect size calculations, $N = 152$ participants (106 female = 70%, 46 male = 30%) with complete datasets could be collected (almost exactly the number of 156 participants recommended for detecting a small effect of $d = 0.20$ in a within *t*-test with *alpha* = 0.050 and *beta* = 0.200). Effect sizes were calculated using G*Power [83,84].

### 5.2. Data collection

***Virtual T-maze task paradigm.*** In our behavioral data collection task, participants navigated through a desktop virtual reality T-maze with five different event types: "negative", "positive", "negative-and-positive", "positive-and-positive", and "ambiguous". In-depth descriptions of this previously established paradigm can be found in prior publications [39–42], which is why we focus on key aspects and provide a related schematic in Fig. 2. After briefing, participants were seated in front of a 61 cm (24″) monitor in 50–60 cm distance and received headphones. Participants started each event looking in the direction of the T-arms. Events were triggered by moving forward and then cued for 4 s by event-specific color cues on the central T-maze wall. After cueing, event-type-specific stimuli occurred, which could be a sheep and/or a monster. Catching sheep was rewarded (credit gain, harmonious sound), being caught by monsters was punished (credit loss, inharmonious sound). Participants were instructed to collect as many credits as possible. Event type order was balanced and randomized so that each event type was delivered 20 times. Each of these 20 trials lasted approximately 13 s. Participants could freely take self-timed breaks after each trial. The task was preceded by a training phase, making sure that the paradigm could be performed successfully. A post-task manipulation check was included, in which participants rated each event type. This led to a total paradigm length of approximately 50 min.

***Electroencephalography (EEG).*** EEG was collected from 64 electrode positions based on the 10-10-system with an Ag/AgCl passive electrode cap (Braincase from https://www.easycap.de/) that was specifically customized for our lab. AFz served as ground electrode, Cz as reference. The exact locations of the further electrode positions of our customized cap setup are provided in Fig. S4 and as distinct black dots in the scalp topography plots of Fig. 1 and Fig. S3. For optimal coupling, we applied abrasive salt-free and hypoallergenic electrolyte-gel (ABRALYT 2000 from https://www.easycap.de/), which allowed us to keep impedances below 5 kOhm. We further utilized the BrainVision products BrainAmp Standard and Recorder 1.20 software (https://www.brainproducts.com/). All data were recorded with a sampling rate of 250 Hz including a low cut-off filter of 0.1 Hz.

***Self-report mood scales.*** A paper version of the SAM and digital VAMS were utilized [59,60]. The SAM scales served as global mood indicators ("valence", "arousal"), while the VAMS were more specific ("happy", "sad", "anxious", "angry", "energetic", "tired", "tense", "confused").

***Experimental protocol including TUS.*** For a detailed overview of the experimental session structure of our double-blind within-subjects study see Table 2. Replicating earlier approaches, our TUS setup matched the aforementioned TUS studies on the right PFC [27,28] and used the same specifically manufactured TUS device prototype. For an overview on these studies including our current work and all related TUS parameters see Table 1. For related TUS beam modelling see Fig. 3, which was created using SMART FUS [85] and a representative template brain scan set provided by BrainVoyager (https://www.brainvoyager.com/).

TUS application was carried out by two experimenters, one fixating the transducer and the gel pad to the participant's head and one starting the procedure on the device. The transducer was either applied in the correct orientation (right PFC TUS), so that the ultrasonic beam targeted the skull, or in the exact opposite way (Sham), so that the beam target emanated in the opposite direction, away from the skull. Participants were asked not to move or talk, and experimenters remained silent during the procedure as well, so the audible sensations in the laboratory during this time consisted of the 40 Hz humming of the pulse repetition

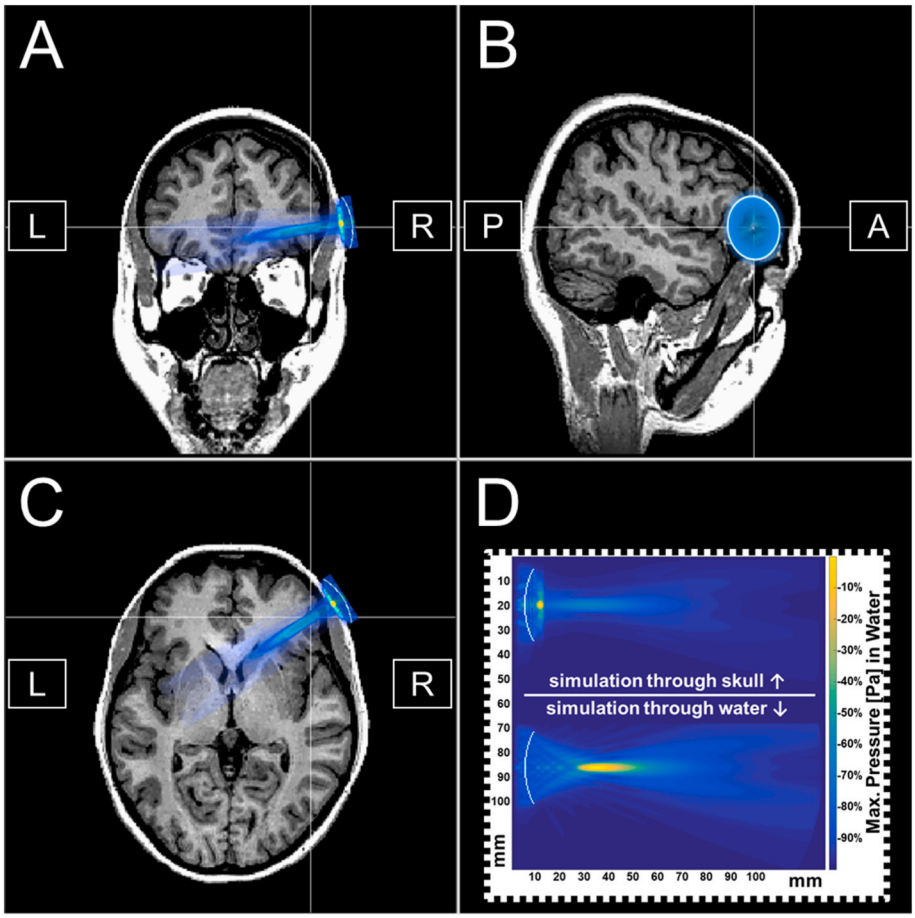


**Fig. 3.** Acoustic beam modelling of our transcranial ultrasound neuromodulation (TUS) targeting the right prefrontal cortex via electroencephalography position F8. Figure originally published in https://psyarxiv.com/g97ky/ (CC-By Attribution 4.0 International). This figure has been created using SMART FUS [85] and a representative template brain scan provided by BrainVoyager (https://www.brainvoyager.com/). For SMART FUS, the following parameters were applied: bone thickness in mm = 3.1, focal depth of the TUS transducer in mm = 30, application angle in degrees = 90, fundamental TUS frequency in kHz = 500, and diameter of the TUS transducer in mm = 30.

frequency (PRF) of our TUS device, which was activated in both the TUS and the Sham condition, plus further humming noises from the laboratory environment (climatization and computer equipment). Since the specifically manufactured TUS device did not allow users to know which side of the transducer was emitting the ultrasonic beam, the experimenters were not aware of which conditions they were executing until they were informed after the entire study was completed, meaning both experimenters were fully blinded until data collection was finished. In addition, the TUS mood effects found in previous studies (which had not been published at the time this study was conducted), were not mentioned to experimenters and participants. They only received the information in the sense of a cover story that we wanted to replicate previous TUS-independent T-maze findings and, as a side question, explore whether ultrasound, as it is used in the context of medical diagnostics, can possibly influence EEG signal measurements, which would have practical implications if both methods would be used at the same time. To strengthen this cover story, participants and experimenters were told that both sides of the specifically manufactured TUS device emitted ultrasonic beams, only distinguished by their different target foci. These two sides of the transducer were distinguished by two differently colored stickers, one in the form of a red spot and one in the form of a blue spot. For each session, the experimenters were instructed which side of the transducer ("red" or "blue") they were meant to direct towards the head. For detailed photographs of the transducer, see Fig. S5.

As part of our briefing at the beginning of our experimental session, all participants were instructed that they should immediately tell us if something felt odd or uncomfortable to them and in our debriefing at the end of our experimental session this was also checked to ensure that participants always felt well during and after our experimental session. Specific questions for potential TUS side effects were avoided, since we did not want to create the impression that we would expect TUS to have any specific effects (and potentially accompanying side effects). No TUS-specific side effects were reported. To some extent, this could have been related to our relatively indirect way of asking for TUS-specific side effects. However, it should be noted that our participants were not shy to report side effects in other contexts: Our study included a familiarization session without TUS or Sham that allowed participants to get used to our paradigm and laboratory in general (as mentioned in Table 2). Based on this familiarization session, nine participants that would have been potentially eligible did decide not to take part in our TUS versus Sham sessions since they felt uncomfortable, either due to simulator sickness caused by moving in the virtual T-maze or due to feelings of sore skin caused by the EEG setup. In addition, five potential participants did not participate because they were not comfortable with the paradigm in general and two potential participants did decide not to participate because of the basic information that was given on the transcranial ultrasound as a relatively new research method at the familiarization session briefing. This might have led to filtering out relatively more sensitive participants and might thus contribute to explaining the absence of TUS-specific side effect reports.

## Data availability statement, related preregistration, and related preprint

Data and statistical analyses are available from the corresponding author on reasonable request. They will also be made available on the Open Science Framework (OSF) when all analyses in the context of this preregistration (https://osf.io/9fqkz?view_only=edef266d57c14c3aa3395fc740c66dd8, including further subprojects) have been finalized. A related preprint can be found on https://psyarxiv.com/yvmsp/.

## CRediT authorship contribution statement

Philipp Ziebell: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration. Johannes Rodrigues: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing – Review & Editing, Project Administration, Funding Acquisition. André Forster: Methodology, Writing – Review & Editing, Visualization. Joseph L Sanguinetti: Methodology, Resources, Writing – Review & Editing. John JB Allen: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision. Johannes Hewig: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding Acquisition.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: Joseph L Sanguinetti is paid a salary and is a shareholder in Sanmai Technologies, PBC. Philipp Ziebell, Johannes Rodrigues, André Forster, John JB Allen, and Johannes Hewig have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2023.08.011.

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