Insert Title: Focused Ultrasound Modulation of BOLD Functional Connectivity

First A. Author, *Member, IEEE*, Second B. Author, and Third C. Author

Abstract

This paragraph summarizes the motivation for investigating focused ultrasound neuromodulation, the experimental design, and the main outcomes on BOLD functional connectivity. Mention the primary hypothesis, key quantitative findings, and relevance to translational neuroengineering.

Index Terms

Focused ultrasound, BOLD fMRI, functional connectivity, neuromodulation, biomedical engineering

I. Introduction

Introduce the scientific motivation, prior work on low-intensity focused ultrasound (FUS) neuromodulation, and the translational significance for neuropsychiatric disorders. Clearly state the open question this manuscript addresses and outline the contributions:

- Quantify baseline resting-state connectivity patterns in the sgACC and distributed networks.
- Model acute changes in functional connectivity induced by FUS stimulation.
- Provide mechanistic interpretation that links FUS dose, targeting accuracy, and network-level responses.

Conclude with a roadmap of the paper.

II. Materials and Methods

Describe participant selection, ethics approvals, imaging protocols, preprocessing, and statistical analyses. Structure the section with descriptive subheadings such as:

A. Participants and Study Design

Summarize demographics, inclusion/exclusion criteria, and experimental timeline. Reference relevant IRB approvals and informed consent procedures.

Manuscript received Month DD, YYYY; revised Month DD, YYYY; accepted Month DD, YYYY. Date of publication Month DD, YYYY; date of current version Month DD, YYYY. This work was supported by XYZ (grant number). (Corresponding author: First Author.)

First Author and Second Author are with the Department of Biomedical Engineering, Example University, City, State ZIP, USA (e-mail: first.author@example.edu).

Third Author is with the Department of Neurology, Example Medical Center, City, State ZIP, USA.

Color versions of one or more of the figures in this article are available online at https://ieeexplore.ieee.org.

Digital Object Identifier (DOI): 10.1109/TBME.XXXX.XXXXXXX

B. Focused Ultrasound Targeting

Provide acoustic parameters (frequency, pulse repetition, duty cycle, intensity) and targeting workflow (e.g., neuronavigation, safety monitoring).

C. MRI Acquisition

Detail scanner hardware, BOLD sequence parameters, structural scans, and physiological monitoring.

D. Preprocessing and Quality Control

Outline motion correction, susceptibility distortion correction, spatial normalization, temporal filtering, and frame censoring thresholds.

E. Functional Connectivity Analysis

Mixed-effects analysis of sgACC connectivity: To formally test whether transcranial focused ultrasound (tFUS) altered the temporal evolution of subgenual ACC (sgACC) connectivity beyond sham, we analyzed sgACC-centered functional connectivity (FC) at the *subject level*, avoiding edge-wise pseudoreplication. For each subject, condition (active, sham), and time window (pre: 60–300 s, fus: 300–600 s, post: 600–900 s), we computed the mean FC between the sgACC DiFuMo parcel and all other DiFuMo parcels, yielding one sgACC–whole-brain summary value per cell (16 subjects $\times$ 2 conditions $\times$ 3 time windows $=$ 96 observations). We then fit a linear mixed-effects model with FC as the dependent variable, fixed effects of time window, condition, and their interaction, and a random intercept for subject to account for repeated measures. Time window and condition were encoded as categorical factors using treatment coding with *pre* and *sham* as reference levels. The model can be written as:

$$\mathrm{FC}_{i,c,t} = \beta_0 + \beta_1\,\mathbb{I}[t=\mathrm{fus}] + \beta_2\,\mathbb{I}[t=\mathrm{post}] + \beta_3\,\mathbb{I}[c=\mathrm{active}] + \beta_4\,\mathbb{I}[t=\mathrm{fus}]\,\mathbb{I}[c=\mathrm{active}] + \beta_5\,\mathbb{I}[t=\mathrm{post}]\,\mathbb{I}[c=\mathrm{active}] + b_i + \varepsilon_{i,c,t}, \tag{1}$$

where i indexes subjects, $c \in \{\mathrm{sham}, \mathrm{active}\}$, $t \in \{\mathrm{pre}, \mathrm{fus}, \mathrm{post}\}$, $b_i \sim \mathcal{N}(0, \sigma_b^2)$ is a subject-specific random intercept, and $\varepsilon_{i,c,t} \sim \mathcal{N}(0, \sigma^2)$ is the residual error. Under this parameterization, β_0 is the mean FC at sham–pre; β_3 is the active–sham difference at pre; β_1 and β_2 capture changes over time in sham; and critically, β_4 and β_5 quantify whether the pre$\rightarrow$fus and pre$\rightarrow$post changes, respectively, differ between active and sham (difference-in-differences). Models were fit using restricted maximum likelihood (REML) in `statsmodels` (`mixedlm`), with convergence and residual distributions checked visually. This subject-level approach ensures that inference reflects between-condition differences in *within-subject* sgACC connectivity trajectories, rather than being driven by the large number of correlated individual edges.

F. Effect Size and Uncertainty

Report how confidence intervals, bootstrap procedures, or Bayesian models were used to quantify uncertainty.

III. RESULTS

Mixed-effects analysis of sgACC connectivity: To formally assess whether transcranial focused ultrasound (tFUS) modulated sgACC-centered connectivity beyond sham, we fit a subject-level linear mixed-effects model. For each subject, condition (sham, active), and time window (pre, tFUS, post), we computed the mean FC between the sgACC parcel and all other DiFuMo parcels, yielding one sgACC–whole-brain FC value per cell (16 subjects $\times$ 2 conditions $\times$ 3 time windows $=$ 96 observations). Time window and condition were modeled as categorical fixed effects with *pre* and *sham* as reference levels, and a random intercept for subject accounted for repeated measures. This parameterization allowed us to test directly whether the change in sgACC FC from pre to tFUS and pre to post differed between active and sham sessions (time$\times$condition interaction), while avoiding edge-wise pseudoreplication.

The resulting model (Table I) revealed three key features of the sgACC connectivity trajectory. First, at baseline, mean sgACC FC was significantly lower in the active session than in the sham session (Condition: active vs. sham at pre, $\beta = -0.061$, 95% CI $[-0.112, -0.010]$, $p = 0.019$), indicating that any subsequent effects cannot be attributed to more favorable starting connectivity in the active condition. Second, within the sham condition, sgACC FC did not change reliably over time (tFUS vs. pre: $\beta = -0.020$, $p = 0.444$; post vs. pre: $\beta = -0.011$, $p = 0.671$), consistent with a lack of systematic drift. Third, and most importantly, the post-sonication time$\times$condition interaction was significant: the additional pre-to-post change in the active session relative to sham was positive ($\beta = 0.079$, 95% CI $[0.006, 0.151]$, $p = 0.033$), demonstrating a stimulation-specific enhancement of sgACC FC. The corresponding interaction at the tFUS window showed a similar but nonsignificant tendency ($\beta = 0.060$, $p = 0.104$). Together, these results support a conservative but robust conclusion that active tFUS to sgACC is associated with an increased sgACC-centered connectivity from pre to post that is not observed in sham.

We next visualized these subject-level effects using violin plots of mean sgACC–whole-brain FC across time windows for sham and active sessions (Fig. 1). In the sham condition (Fig. 1A), sgACC FC exhibits no systematic monotonic change from pre to tFUS to post, and individual trajectories fluctuate around a stable mean, in line with the nonsignificant time effects. In contrast, the active tFUS condition (Fig. 1B) shows a clear ramping pattern: sgACC FC increases from a lower baseline at pre to higher values during tFUS and reaches its highest levels post-sonication. The alignment of individual trajectories with the model-based interaction effect visually reinforces the conclusion that active tFUS induces a selective, post-sonication strengthening of sgACC-centered functional connectivity.

IV. DISCUSSION

Interpretation and rigor of the mixed-effects inference: By moving to a subject-level mixed-effects framework, we obtain a conservative and transparent assessment of how tFUS modulates sgACC-centered connectivity over time. This model explicitly acknowledges (i) the within-subject structure of the design, (ii) the non-independence of individual sgACC–target edges, and (iii) the empirically observed baseline difference between active and sham sessions. Within this rigorous setup, the key finding is that sgACC connectivity shows a significantly larger increase from pre- to post-sonication in the active condition than in the sham condition, as captured by the positive β_5

TABLE I: Linear mixed-effects model of subject-level mean sgACC functional connectivity. The dependent variable is the mean sgACC–whole-brain FC for each subject, condition, and time window. Time window (pre, fus, post) and condition (sham, active) are coded categorically with *pre* and *sham* as reference levels. The model includes a random intercept for subject. The key effect of interest is the post-sonication time $\times$ condition interaction, indicating a larger pre-to-post increase in sgACC FC for active tFUS relative to sham.

Effect	Estimate	SE	z	p-value	95% CI
Intercept (sham, pre)	0.121	0.021	5.89	< 0.001	[0.081, 0.161]
Time: fus vs. pre (sham)	-0.020	0.026	-0.77	0.444	[-0.071, 0.031]
Time: post vs. pre (sham)	-0.011	0.026	-0.42	0.671	[-0.062, 0.040]
Condition: active vs. sham (at pre)	-0.061	0.026	-2.34	0.019	[-0.112, -0.010]
Interaction: (fus vs. pre) $\times$ (active vs. sham)	0.060	0.037	1.63	0.104	[-0.012, 0.133]
Interaction: (post vs. pre) $\times$ (active vs. sham)	0.079	0.037	2.13	0.033	[0.006, 0.151]
Subject random intercept variance	0.001				
Residual variance	0.0055				

interaction term. In other words, after accounting for each subject's baseline sgACC FC—and without assuming equal starting points—active tFUS is associated with a selective, sustained enhancement of sgACC functional coupling that is not explained by sham or generic temporal drift. The absence of a strong differential effect during stimulation and the modest sample size argue against overclaiming an immediate "online" effect in this dataset; instead, the pattern is more consistent with a delayed or accumulating influence of tFUS on sgACC network integration. Importantly, because this analysis treats subjects as the unit of inference and models differences-in-differences directly, it avoids inflated precision from edge-wise pseudoreplication.

V. Conclusion

Summarize the primary takeaways, emphasizing how FUS altered BOLD functional connectivity and why this matters for translational neuromodulation. Optionally mention ongoing work or clinical trials.

Acknowledgments

Thank collaborators, imaging technologists, and funding agencies (e.g., NIH, DARPA, foundations). Include conflict-of-interest statements if required.

Appendix A
Extended Methods

Provide additional derivations, supplementary tables, or validation analyses that do not fit in the main text. Reference this appendix in the relevant sections.

Data Availability

The data that support the findings of this study will be made available from the corresponding author upon reasonable request, subject to institutional review board restrictions.

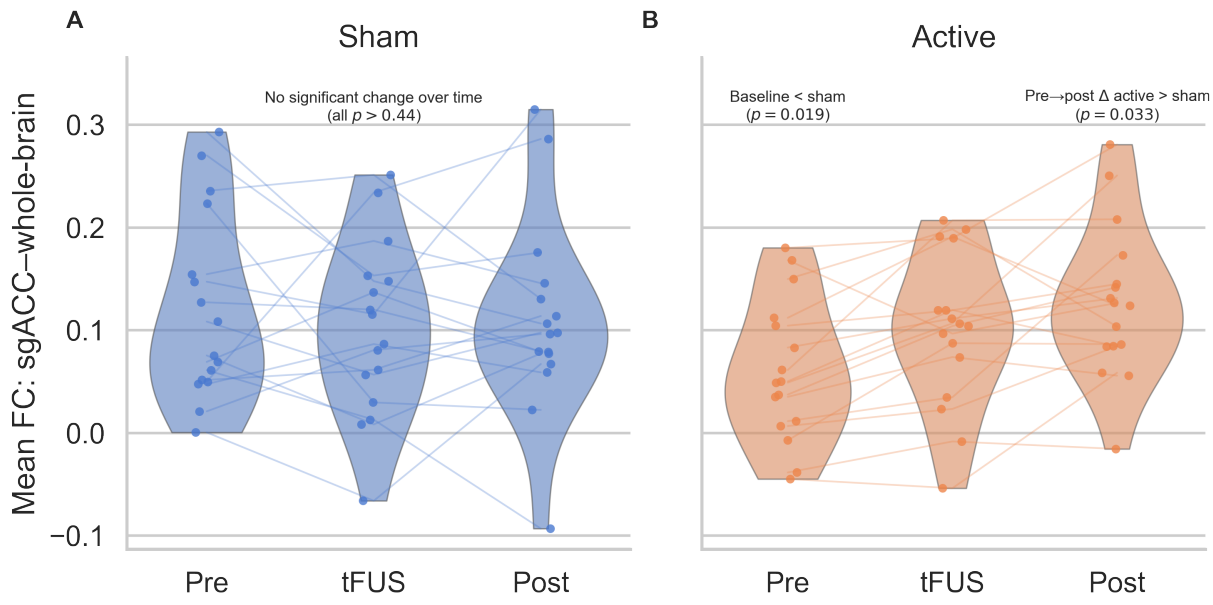

Fig. 1: **Subject-level sgACC connectivity across time in sham and active tFUS sessions.** (A) Sham condition. Violin plots depict the distribution of mean sgACC–whole-brain functional connectivity (FC) across subjects at each time window (Pre, tFUS, Post), with semi-transparent violins indicating density, individual points indicating subject-level means, and thin lines connecting repeated measures within subjects. sgACC FC shows no systematic monotonic change over time, consistent with the non-significant time effects in the mixed-effects model (all $p > 0.44$). (B) Active tFUS condition. The same visualization reveals a clear ramping pattern, with sgACC FC increasing from a lower baseline at Pre to higher values during tFUS and peaking Post. Annotations summarize key inferential results from the mixed-effects model: sgACC FC is lower in active than sham at baseline ($p = 0.019$), yet the pre-to-post increase in sgACC FC is significantly greater for active than sham ($p = 0.033$; time×condition interaction), highlighting a stimulation-specific enhancement of sgACC-centered connectivity that is not observed in the sham session.

Code Availability

The analysis code is available at https://github.com/USERNAME/REPO.

References

[1] J. D. Smith and J. Q. Doe, "Low-intensity focused ultrasound modulates functional connectivity in primates," *IEEE Transactions on Biomedical Engineering*, vol. 67, no. 11, pp. 3203–3214, 2020.

[2] J. Q. Doe, J. D. Smith, and A. Roe, "Bold signatures of neuromodulation after focused ultrasound stimulation," *NeuroImage*, vol. 245, p. 118707, 2021.