RUNNING TITLE: FOCUSED ULTRASOUND AMYGDALA NEUROMODULATION

Supplementary Materials for:

Low-Intensity Transcranial Focused Ultrasound Amygdala Neuromodulation: A Double-Blind Sham-Controlled Target Engagement Study and Unblinded Single-Arm Clinical Trial

Bryan R. Barksdale MD PhD, Lauren Enten BSA, Annamarie DeMarco BS, Rachel Kline BA, Manoj K. Doss PhD, Charles B. Nemeroff MD PhD, and Gregory A. Fonzo, PhD*

Department of Psychiatry and Behavioral Sciences, The University of Texas at Austin Dell Medical School, Austin, TX, USA

*Corresponding Author's Contact Information:

Gregory A. Fonzo, Ph.D.

Health Discovery Building (HDB)

1601 Trinity St., Bldg. B, Z0600

Austin, TX 78712

512-495-5296

gfonzo@austin.utexas.edu

Supplementary Table 1. Study Exclusion Criteria

Exclusion Criterion	Applicable To
Contraindications to undergoing transcranial focused ultrasound (tFUS) and/or magnetic resonance imaging (MRI), including irremovable internal ferrous metals, implants, pacemakers, shunts, or valves.	MATRD, HC
Organic neurological conditions including history of stroke, brain tumors or hemorrhages, penetrating head injuries, moderate-to-severe traumatic brain injuries, or brain surgery.	MATRD, HC
History of seizures (except febrile seizures in childhood).	MATRD, HC
Uncontrolled medical conditions likely to interfere with study participation and/or contribute to confounding effects on mental health symptoms.	MATRD, HC
If female, pregnant or planning to become pregnant during the course of study participation.	MATRD, HC
Current active suicidal/homicidal ideation, or suicidal/homicidal ideation deemed by the investigators to need immediate clinical attention.	MATRD
Recent (past 3 months) suicide attempt.	MATRD
Current non-alcohol substance use disorder or alcohol use disorder of more than mild severity.	MATRD
Current or recent (past 3 months) psychotic symptoms.	MATRD
Meeting current diagnostic criteria for a manic episode.	MATRD
Active engagement in one or more evidence-based or experimental treatments likely to confound experimental effects. • General supportive therapy without evidence-based interventions (e.g., cognitive restructuring, behavioral activation, exposure therapy, etc.) allowable if participants maintained the same frequency of visits over the course of study participation. • If currently on a stable regimen/dosage (no changes within 3 months of enrollment) of psychiatric medication, participants permitted to enroll in study if they agreed to maintain same regimen/dosage throughout duration of study participation (approximately 1-2 months).	MATRD

HC = healthy comparison group; MATRD = mood, anxiety, and trauma-related disorder group

Supplementary Table 2. Secondary Clinical Outcome Measures

Measure Name	Measured Construct
Anxiety Sensitivity Index-3 (ASI)	Anxiety sensitivity
Alcohol Use Disorders Identification Test (AUDIT)	Alcohol use disorder
Beck Anxiety Inventory (BAI)	Anxiety symptoms
Beck Depression Inventory-II (BDI)	Depression symptoms
Connor-Davidson Resilience Scale (CDRISC)	Resilience
Daily Drinking Questionnaire Revised (DDQR)	Alcohol use patterns
Generalized Anxiety Disorder-7 (GAD-7)	Generalized anxiety disorder
Mood and Anxiety Symptom Questionnaire Anxious Arousal (MASQ-AA)	Anxious arousal
Mood and Anxiety Symptom Questionnaire Anhedonic Depression (MASQ-AD)	Anhedonia
Patient Health Questionnaire-9 (PHQ-9)	Depression symptoms
Pittsburgh Sleep Quality Index (PSQI)	Sleep quality/insomnia
Positive and Negative Affect Scale Short Form Positive Affect (PANAS-PA)	Positive affect
Positive and Negative Affect Scale Short Form Negative Affect (PANAS-NA)	Negative affect
PTSD Checklist for DSM-5 (PCL-5)	PTSD symptoms
Quick Inventory of Depressive Symptomatology Self Report (QIDS)	Depression symptoms
Snaith Hamilton Pleasure Scale (SHAPS)	Anhedonia
Spielberger State Trait Anxiety Inventory State Anxiety (STAI-S)	State anxiety
Spielberger State Trait Anxiety Inventory Trait Anxiety (STAI-T)	Trait anxiety
Temporal Experiences of Pleasure Scale Anticipatory Pleasure (TEPS-AP)	Hedonic capacity in anticipation of reward
Temporal Experiences of Pleasure Scale Consummatory Pleasure (TEPS-CP)	Hedonic capacity in receipt of reward
Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency Impulsive Behavior Scale Short-Form (S-UPPS-P)	Impulsivity
World Health Organization Quality of Life BREF Global Health (WHO-QOL Global)	Overall global health
World Health Organization Quality of Life BREF Environmental Health (WHO-QOL Envir)	Environmental health
World Health Organization Quality of Life BREF Physical Health (WHO-QOL Physical)	Physical health
World Health Organization Quality of Life BREF Psychological Health (WHO-QOL Psych)	Psychological health
World Health Organization Quality of Life BREF Social Health (WHO-QOL Social)	Social health

Supplementary Table 3. MRI-Guided Targeting of Brainsonix Ultrasound Transducer.

Step	Description
1	Deposit participant in MRI scanner.
2	Collect high-resolution T1-weighted anatomical image.
3	Use T1 image and scanner measurement tools to gauge target focal depth of transducer, e.g., 55mm or 65mm focal depth.
3	Pull T1-weighted image off scanner computer and enter into neuronavigation system software for construction of individualized head model. Run T1-weighted anatomical through head model construction process.
4	Remove participant from MRI bore, escort to MRI control room, and affix transducer to left temporal window (or desired initial placement site) using transducer holder and velcro head straps.
5	Escort participant back into scanner room, place in MRI bore/head coil, and acquire a T1-weighted anatomical scout image, e.g., 3D Fast Low Angle Shot with non-selective excitation, sagittal, TR/TE = 3.20/1.37 ms, slice thickness = 1.6 mm, flip angle = 8 deg, FOV = 260 mm, matrix size = 128 x 160 x 160, voxel size = 1.6 x 1.6 x 1.6 mm, duration = 0:17.
6	Use T1-weighted scout image to visualize T1-sensitive concentric rings on back of transducer. Open image in 3-dimensional viewer and place crosshairs in the coronal plane in the center of the transducer concentric rings.
7	Adjust axes of viewer in the coronal and axial directions, such that y-axis in both planes is parallel with face of transducer and x-axis is perpendicular.
8	Use scanner measurement tool to draw/measure a line from the face of the transducer along the perpendicular x-axis to the target focal depth of the transducer (i.e., a 65 mm line for a 65 mm focal depth transducer). The end of the line is the approximate location of the ultrasound focus at the current placement.
9	If the end of the line does not lie at the desired anatomical target, use scanner measurement tools to estimate the offset of the line in the axial and coronal planes from an approximately parallel line that would terminate in the desired anatomical target. Write down these offsets. If the line approximating line of site does terminate in the desired anatomical target, skip to step #13.
10	Remove participant from the scanner bore, and adjust placement of transducer on the side of the head to match the displacements noted with the scanner measurement tool, e.g., if the current line of site is 1cm inferior to the desired target in the coronal plane, move the transducer up about 1cm on the y-axis in the coronal plane (top-to-bottom of head). Repeat the same movement in the y-axis of the axial plane (front-to-back of head) to match noted displacement.
11	Place participant back in MRI bore and collect another T1-weighted anatomical scout.
12	Repeat steps 6-10 as needed.
13	Once proper placement is verified, remove participant from the MRI scanner bore and escort them into MRI control room.
14	Affix neuronavigation coil tracker to transducer (while still affixed to participant's head at optimized location) and neuronavigation head tracker to participant's head.
15	Calibrate both the coil tracker and head tracker in the software using typical protocol (e.g., nasion/inion, head-shape points, etc.)
16	Once calibrated, use manual button box or other method to mark location of ultrasound transducer by sending a TTL pulse to neuronavigation software.
17	Be sure to save/export location marker of delivered TTL pulse at neuronavigated location for use in future neuronavigation targeting settings.
18	Remove neuronavigation coil tracker, head tracker, and focused ultrasound transducer from participant. Clean and stow equipment for future use.

Supplementary Table 4. Detailed Information on Study Clinical Sample

Study ID	Completed?	Age	Sex	Race	Ethnicity	Education	Diagnoses	Medications	Current Therapy
OM_AD002	Yes	22	F	W/C	NH	4-yr degree	BD2, GAD, PTSD	lamotrigine, quetiapine, and lithium	Yes
OM_AD003	Yes	20	F	W/C	NH	HS Grad	MDD, PD, SAD	None	No
OM_AD005	Yes	21	F	W/C	H	4-yr degree	BD2, AUD (mild)	None	No
OM_AD006	Yes	28	M	W/C	NH	Some college	MDD, PTSD	lithium 900mg	No
OM_AD009	Yes	21	F	W/C	H	Some college	MDD	escitalopram 20mg	No
OM_AD011	Yes	40	F	W/C	NH	Doctorate	GAD, OCD	fluoxetine 60mg, buproprion 300mg	No
OM_AD012	Yes	18	F	B/AA	NH	2-yr degree	MDD	fluoxetine 20mg	No
OM_AD013	Yes	18	F	W/C	H	2-yr degree	BD1, PTSD	None	No
OM_AD014	Yes	18	F	W/C	H	HS grad	PTSD	None	No
OM_AD015	Yes	18	M	W/C NA/AI/AN	NH	Some college	MDD, PTSD	None	No
OM_AD016	Yes	20	M	W/C	H	Some college	MDD, SAD, GAD, OCD	buproprion 300mg	No
OM_AD017	Yes	32	M	W/C	H	Doctorate	MDD, GAD	venlafaxine 150mg, clonazepam 0.5mg	No
OM_AD019	Yes	19	M	A	NH	2-yr degree	GAD, SAD	escitalopram 20mg	Yes
OM_AD020	Yes	26	F	W/C	NH	4-yr degree	GAD	trazodone 50mg-100mg as needed	Yes
OM_AD021	Yes	20	F	W/C	H	Some college	BD1, GAD, PTSD	None	Yes
OM_AD022	Yes	42	F	W/C	NH	2-yr degree	MDD, PTSD	buproprion 300mg, lurasidone, gabapentin	No
OM_AD023	Yes	19	M	W/C	NH	Some college	GAD, SAD, PTSD	venlafaxine 150mg	No
OM_AD024	Yes	39	F	NA/AI/AN	H	Prof degree	MDD, GAD, PTSD, AUD	None	No
OM_AD025	Yes	19	F	W/C	NH	HS grad	GAD	sertraline 200mg	No
OM_AD028	Yes	21	M	W/C	H	Some college	BD1, GAD, SAD, OCD	None	No
OM_AD029	Yes	18	F	A	NH	Some college	BD1, PD, GAD, PTSD	None	Yes
OM_AD001	No	21	F	W/C	H	4-yr degree	BD2, SAD, AUD (ER)	duloxetine 60mg	No
OM_AD004	No	21	M	A	NH	Some college	BD2, CUD (ER), SUD (ER), HUD (ER)	None	No
OM_AD007	No	42	F	W/C	NH	Prof degree	MDD	None	Yes
OM_AD008	No	19	M	W/C	H	HS grad	BD2, OCD	escitalopram 25mg, lamotrigine 50mg, naltrexone	Yes
OM_AD010	No	32	F	W/C	NH	Prof degree	GAD	fluoxetine 40mg, buproprion 300mg	Yes
OM_AD018	No	23	F	W/C	H	4-yr degree	MDD, GAD, SAD	fluoxetine 80mg, desvenlafaxine 50mg, lisdexamfetamine 50mg	Yes
OM_AD026	No	22	F	W/C	H	Some college	BD2, GAD, AUD (mild)	lithium 1200mg and lamotrigine 150mg	Yes
OM_AD027	No	21	F	W/C	NH	Some college	MDD, GAD	desvenlafaxine 50mg	Yes

A=Asian; AUD=alcohol use disorder; B/AA=Black/African-American; BD1=bipolar I disorder; BD2=bipolar II disorder; CUD=cannabis use disorder; ER = early remission; F=female; GAD=generalized anxiety disorder; H=Hispanic; HS Grad = high school graduate; HUD=hallucinogen use disorder; M=male; MDD=major depressive disorder; NA/AI/AN=Native American/American Indian/Alaskan Native; NH=non-Hispanic; OCD=obsessive-compulsive disorder; Prof degree=non-doctoral post-baccalaureate professional degree; PD=panic disorder; PTSD=posttraumatic stress disorder; SAD=social anxiety disorder; SUD=stimulant use disorder; W/C=White/Caucasian; yr=year.

Supplementary Table 5. Participant Guesses and Confidence Ratings for Active/Sham Sonication following tFUS/fMRI Sessions

MATRD participants

Session 1

		Participant Guess: Active	Participant Guess: Sham
True Condition: Active		7 (4.71 +/- 1.98)	7 (3.57 +/- 2.15)
True Condition: Sham		8 (3.25 +/- 2.92)	5 (5.40 +/- 0.89)

Session 2

		Participant Guess: Active	Participant Guess: Sham
True Condition: Active		8 (5.00 +/- 2.62)	3 (6.33 +/- 1.53)
True Condition: Sham		11 (3.91 +/- 2.66)	3 (5.67 +/- 2.52)

HC participants

Session 1

		Participant Guess: Active	Participant Guess: Sham
True Condition: Active		7 (1.14 +/- 1.57)	5 (3.00 +/- 2.00)
True Condition: Sham		7 (5.71 +/- 3.15)	3 (2.67 +/- 3.06)

Session 2

		Participant Guess: Active	Participant Guess: Sham
True Condition: Active		7 (5.29 +/- 3.15)	3 (2.67 +/- 2.08)
True Condition: Sham		5 (3.20 +/- 2.39)	7 (4.00 +/- 2.05)

Tables depict the 2 x 2 contingency breakdown for participant guesses for participants with mood, anxiety, and trauma-related disorders (MATRD) on the top half and guesses for healthy comparison (HC) participants on the bottom half for active and sham sonication (columns of tables) during each session split by the true condition (rows of tables). Each cell contains the number of guesses for that condition (active or sham) split by the true condition of delivery, and the mean and standard deviation of confidence ratings of those guesses are included in parentheses in each cell next to the total number of guesses. Confidence ratings were made on a 11-point Likert scale ranging from 0 to 10, with 0 being "Not at all confident" and 10 being "Extremely confident."

Supplementary Table 6. Effects of Active vs. Sham Transcranial Focused Ultrasound on fMRI BOLD Signal and Context-Dependent Left Amygdala Connectivity and Differences by Patient Group

Hem.	MNI Atlas Region(s)	Voxels	X	Y	Z	Voxel Stats		Predicted Value (mean, SD)			
						pTFCE Z		MATRD		HC	
						Mean	*SD*	Active	Sham	Active	Sham
Main Effect of Active vs. Sham on Activation, ROI-Constrained Analysis											
L	Hippocampus	121	-36	-17	-16	4.14	0.26	-0.02 (0.02)	0.01 (0.02)	-0.05 (0.03)	0.01 (0.05)
L	Amygdala	26	-29	-2	-25	3.69	0.04	-0.04 (0.02)	-0.01 (0.02)	-0.09 (0.05)	-0.01 (0.05)
Main Effect of Active vs. Sham on Activation, Whole Brain Exploratory Analysis											
L	Thalamus/Pallidum	46	-11	-5	-5	4.99	0.29	-0.03 (0.03)	0.04 (0.03)	-0.03 (0.04)	0.04 (0.04)
L	Insula/Putamen	15	-28	18	-6	4.69	0.07	-0.04 (0.03)	0.01 (0.03)	-0.03 (0.05)	0.03 (0.05)
MATRD vs HC x Active vs. Sham Interaction Effect on Activation, ROI-Constrained Analysis											
R	Hippocampus	16	40	-17	-13	4.29	0.60	0.00 (0.03)	-0.01 (0.04)	-0.04 (0.04)	0.02 (0.04)
MATRD vs HC x Active vs. Sham Interaction Effect on Activation, Whole Brain Exploratory Analysis											
R	Insula	39	40	0	0	4.71	0.07	0.01 (0.03)	-0.05 (0.04)	-0.05 (0.04)	0.01 (0.05)
Main Effect of Active vs. Sham on Left Amygdala gPPI Connectivity, ROI-Constrained Analysis											
R	Superior Frontal Gyrus	10	20	0	50	4.26	0.03	2.27 (0.56)	-2.34 (0.56)	0.74 (0.73)	-1.78 (0.73)
Main Effect of Active vs. Sham on Left Amygdala gPPI Connectivity, Whole Brain Exploratory Analysis											
R	Superior Frontal Gyrus	7	22	0	48	4.80	0.06	2.43 (1.50)	-2.18 (1.50)	2.66 (2.87)	-2.36 (2.87)
MATRD vs HC x Active vs. Sham Interaction Effect on Left Amygdala gPPI Connectivity, ROI-Constrained Analysis											
R	Insula	128	40	4	14	4.63	0.24	2.80 (2.12)	-1.41 (2.06)	-2.40 (2.12)	2.67 (2.54)
MATRD vs HC x Active vs. Sham Interaction Effect on Left Amygdala gPPI Connectivity, Whole Brain Exploratory Analysis											
R	Insula	56	39	6	14	4.93	0.13	3.30 (2.78)	-1.64 (2.49)	-2.52 (2.32)	2.97 (2.93)

X, Y, and Z values are cluster center of mass coordinates in MNI stereotactic space; Voxel stats column depicts the mean and standard deviation of the voxelwise statistics for each effect; Predicted value column lists the summary statistics (mean and standard deviation) by group and condition for each individual's predicted level of activation or connectivity derived from the mixed model; Con = condition effect; gPPI = generalized psychophysiological interaction analysis; GrpxCon = group x condition interaction effect; HC = healthy comparison; Hem = hemisphere; L = left; MATRD = mood, anxiety, or trauma-related disorder; MNI = Montreal Neurological Institute; R = right; ROI = regions of interest; SD = standard deviation.

Supplementary Table 7. Reported Adverse Events

Symptom	Frequency (N)			Severity (0-10)			χ^2	*p*
	A	**S**	**Tx**	**A**	**S**	**Tx**		
Calm feeling	1	2	0	3	4,3	--	0.00	1.00
Decreased concentration	2	1	0	5,7	6	--	0.00	1.00
Tingling in limbs/hands	2	1	0	5,7	1	--	0.00	1.00
Headache	1	1	1	7	2	3	0.00	1.00
Ringing in ears	1	2	0	5	2,4	--	0.00	1.00
Dizziness/Light-headedness	2	1	0	5,4	2	--	0.00	1.00
Vivid memories	0	1	0	--	6	--	0.00	1.00
Unusual sensation in head/face/body	1	2	0	6	3,6	--	0.00	1.00
Uneasiness	1	0	0	6	--	--	0.00	1.00
Irritability	0	0	1	--	--	3	--	--
Total/Mean	**11**	**11**	**2**	**5.45**	**3.54**	**3.00**	0.00	1.00

*Frequency column reports the number of participants in each condition endorsing an adverse event/side effect. Severity column indicates the self-reported severity of the event/symptom for each instance of endorsement, with 0 = not at all noticeable and 10 = extremely noticeable. The χ^2 column indicates the McNemar's test statistic for the difference in observed frequencies of each symptom/event as a function of active or sham focused ultrasound administration in the MRI scanner environment, and the **p** column indicates the p-value for the statistic. A=active focused ultrasound delivered in MRI environment; S=sham focused ultrasound delivered in MRI environment; Tx=active focused ultrasound delivered during daily treatment portion.*

Supplementary Table 8. Sensitivity analyses by psychiatric diagnosis on diagnosis-specific outcomes

Measure	Baseline	PostTx	DOF		ITT		Cohen's *d*
			Num	*Denom*	*F*	*p*	
Major Depressive Disorder (MDD)							
PHQ-9	12.67 ± 4.44	7.56 ± 5.17	1	8.23	14.06	0.005	0.65
SHAPS	4.64 ± 4.15	1.33 ± 3.28	1	9.41	7.49	0.022	0.55
QIDS-SR	11.91 ± 3.53	6.33 ± 4.15	1	10.06	13.41	0.004	0.63
BDI-II	24.00 ± 10.27	12.78 ± 10.95	1	9.95	7.38	0.022	0.52
Generalized Anxiety Disorder (GAD)							
GAD-7	13.13 ± 4.24	9.45 ± 4.32	1	13.18	10.01	0.007	0.90
BAI	24.19 ± 11.00	13.54 ± 9.54	1	11.75	10.37	0.008	0.82
Posttraumatic Stress Disorder (PTSD)							
PCL-5	40.10 ± 16.35	21.50 ± 14.93	1	9.00	15.31	0.004	2.30

Baseline and PostTx columns depict the measure mean ± the standard deviation; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory II; Denom=denominator; DOF=degrees of freedom; GAD-7=Generalized Anxiety Disorder 7; ITT=intent-to-treat; Num=numerator; PCL-5=PTSD Checklist for DSM-5; PHQ-9=Patient Health Questionnaire 9; PostTx=post-treatment; QIDS-SR=Quick Inventory of Depressive Symptomatology Self Report; SHAPS=Snaith Hamilton Pleasure Scale.

Supplementary Table 9. Changes in Amygdala Task Activation Following Repetitive Transcranial Focused Ultrasound

Hem.	MNI Atlas Region(s)	Voxels	X	Y	Z	Voxel Stats		Predicted Value (mean)									
						pTFCE Z		Pre					Post				
						Mean	*SD*	A	F	H	N	S	A	F	H	N	S
Main Effect of Time, ROI-Constrained Analysis																	
R	Amygdala	265	24	-4	-18	5.67	0.44	0.26	0.16	0.15	0.12	-0.07	0.03	0.01	-0.02	0.03	-0.11
L	Amygdala	205	-29	-2	-25	6.92	1.14	0.28	0.20	0.15	0.13	-0.07	0.07	0.04	0.01	0.05	-0.04
Time x Condition Interaction, ROI-Constrained Analysis																	
L	Amygdala	8	-23	-3	-26	3.40	0.02	0.23	0.01	0.09	-0.08	-0.04	0.01	0.03	0.06	0.09	-0.01

X, Y, and Z values are cluster center of mass coordinates in MNI stereotactic space; Voxel stats column depicts the mean and standard deviation of the voxelwise statistics for each effect; Extractions column reports the mixed model parameter and significance value using extracted individual cluster beta values for each subject; Predicted value column lists the mean predicted value by time point and condition for each individual's predicted level of activation derived from the mixed model; A = Angry Faces; Con = condition effect; F = Fearful Faces; H= Happy Faces; Hem = hemisphere; L = left; MNI = Montreal Neurological Institute; N = Neutral Faces; Post = post-treatment; Pre = pre-treatment; R = right; ROI = regions of interest; S= Shape Processing Comparator; Time x Con = time x condition interaction effect.

Supplementary Figure 1. Step-by-Step Process for Visually Distinguishing and Localizing the Center of the Left Amygdala at the MRI Scanner Using T1-weighted Images

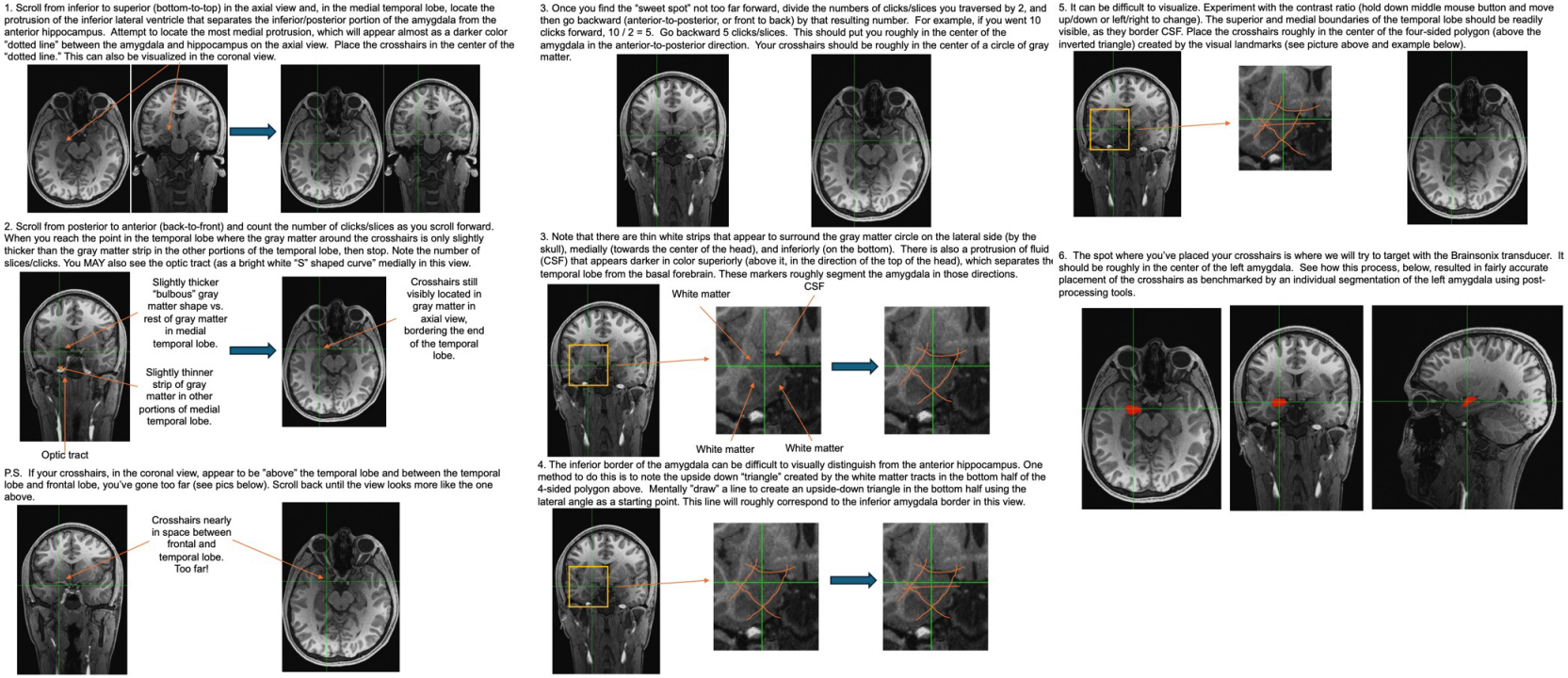


Figure depicts the step-by-step process developed for this study and used by scanner operators to visually distinguish and locate the center of the left amygdala on T1-weighted anatomical images, which was used to guide placement of the Brainsonix transducer for line-of-sight-based targeting towards the center of the left amygdala.

Supplementary Figure 2. Schematic of the Emotional Face Processing Task

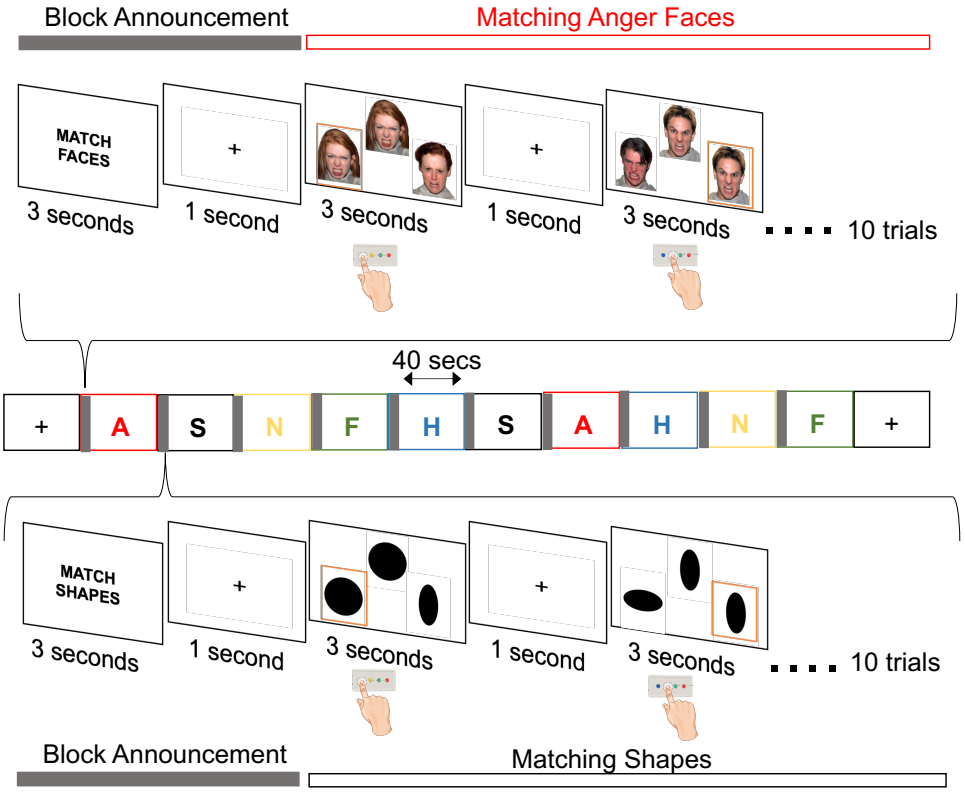


Figure portrays key components of the emotional face processing task used to probe amygdala activity before and after repetitive transcranial focused ultrasound (rtFUS). Order of blocks for one version of the task paradigm is depicted from left-to-right in the center of the figure, with conditions color-coded and labeled by first letter (A=anger (red); F=fear (green); H=happy (blue); N=neutral (yellow); S=shapes (black); +=pre/post-task fixation condition). Pop-outs on top depict the structure within a face-matching task block, which begins with a 3-second announcement screen ("Match Faces"), followed by a 1-second fixation cross. Trials of face trios are then presented for 3 seconds, and the participant indicates which of the two faces on the bottom match the face on the top (first button for lower left face, second button for lower right face). Faces then disappear and are followed by another 1-second fixation cross prior to the start of the next trial. All faces were of uniform emotional expression and gender within each trial. All face trials were of uniform emotion within each block. Blocks lasted for 40 seconds in total and were comprised of 10 4-second trials, not including the 4-second announcement screen at the start of each block. Each condition was represented over 2 non-contiguous blocks, for 20 trials of each condition in total. Pop-out on the bottom indicates content of one of the shape-processing blocks, which is procedurally similar but involves matching circles/ovals rather than faces.

Supplementary Figure 3. CONSORT Diagram

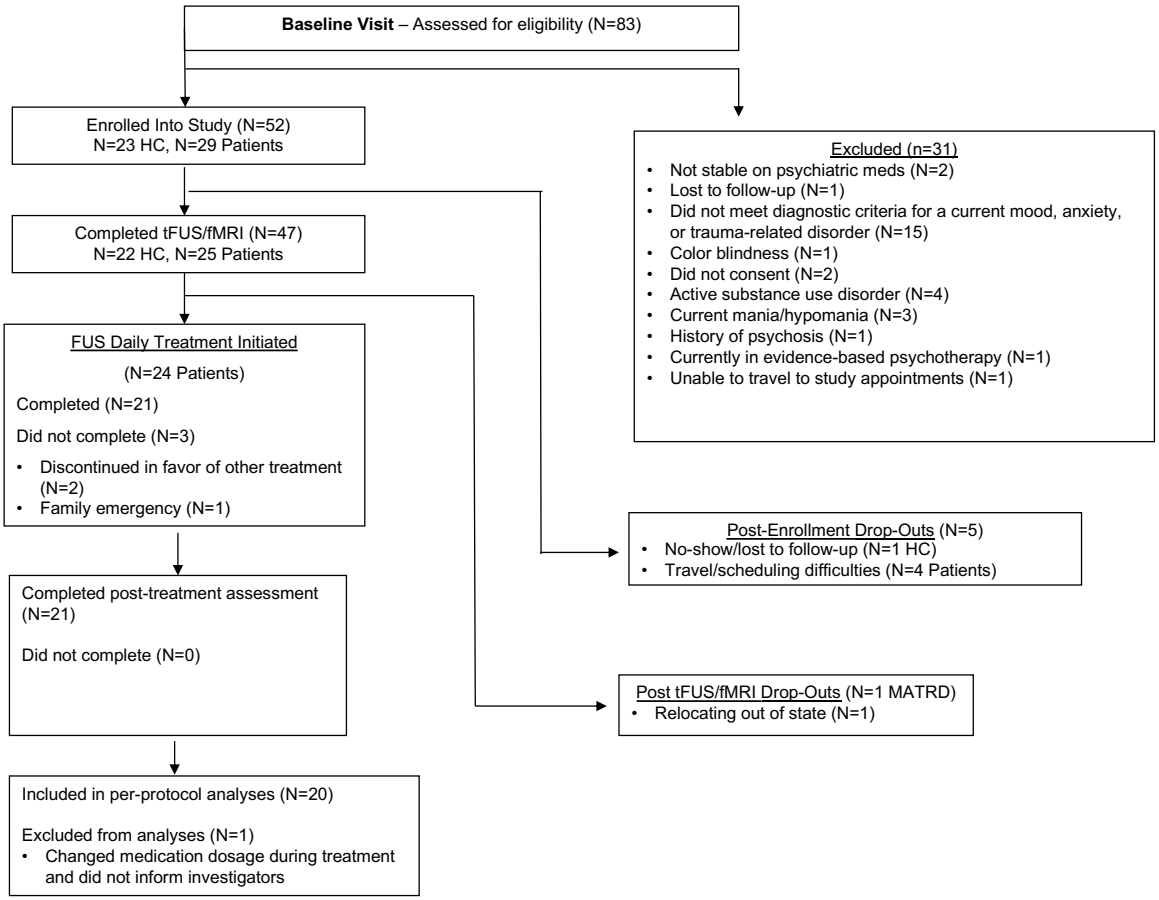


Figure depicts the flow of participants through consent/baseline assessment, enrollment, and the various phases of study completion, with reasons and numbers for dropouts noted.

Supplementary Figure 4. Psychophysiological Interaction Analysis Testing Main Effect of Active vs. Sham tFUS on Left Amygdala Context-Dependent Connectivity and Its Interaction with Patient Group.

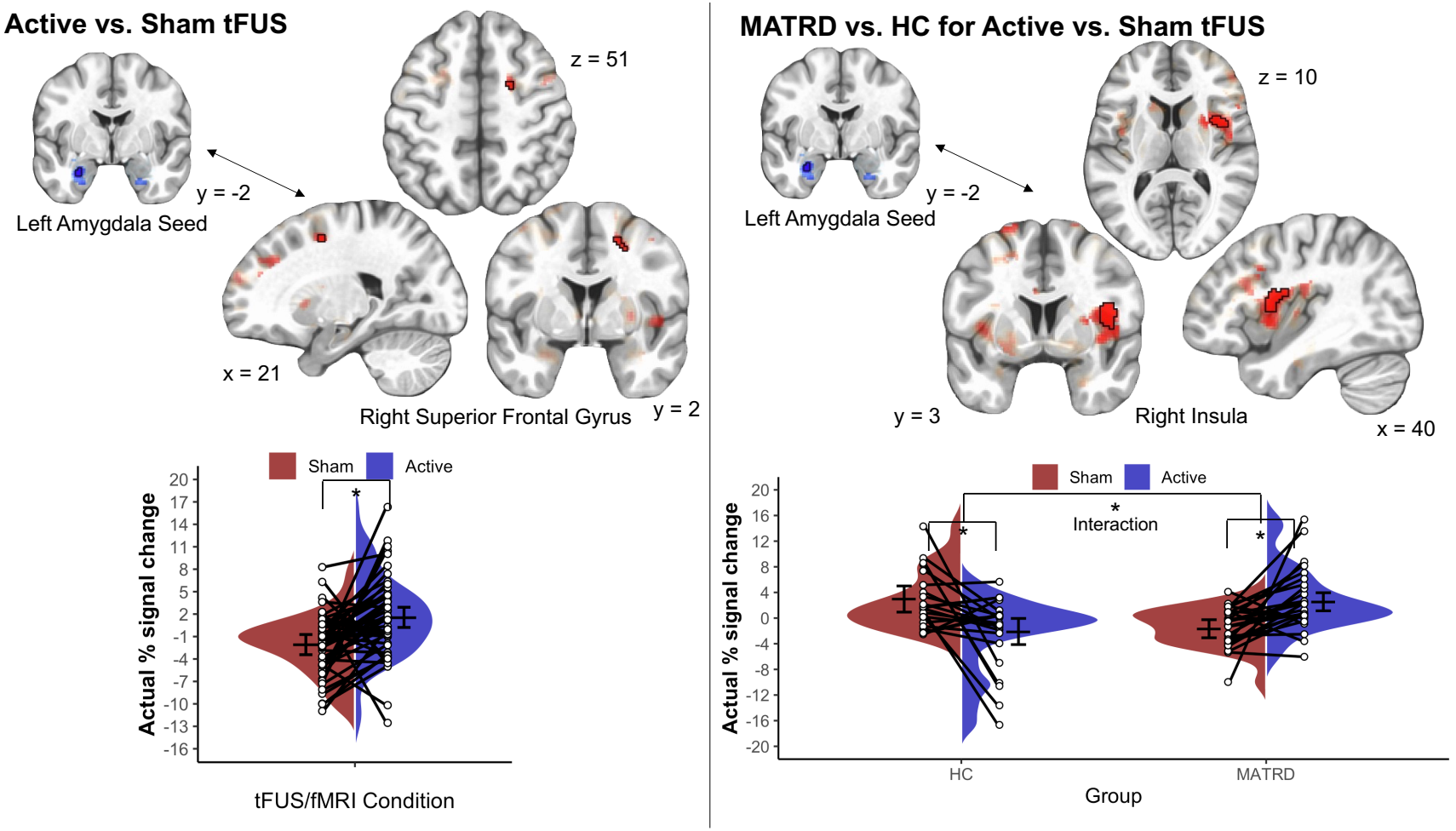


Figure depicts brain areas displaying probabilistic threshold-free cluster enhancement corrected significant effects from the limbic region of interest constrained analysis of the voxelwise whole-brain linear mixed effects model for the main effect of active vs. sham transcranial focused ultrasound (tFUS)(left panel) and the interaction effect of MATRD vs. HC group differences for active vs. sham tFUS (right panel) for left amygdala context dependent (tFUS delivered vs. not delivered) connectivity. Areas of significant difference in evoked left amygdala connectivity changes for active vs. sham tFUS and its interaction with patient group are depicted as colorized and signed based upon the contrast of active vs. sham tFUS mean left amygdala connectivity values at each voxel (main effect) and the mood, anxiety, and trauma-related disorder (MATRD) patient group vs. healthy control differences for active vs. sham tFUS effects (interaction effect) at each voxel. Therefore, warm (light to deep red) colors indicate regions of significantly larger left amygdala connectivity for active vs. sham tFUS (on vs. off) and, for the

interaction with group, greater increases/lesser decreases in left amygdala connectivity for MATRD patients vs. HC participants for active vs. sham tFUS. Suprathreshold voxels are outlined in black with a linear fade applied to subthreshold voxels as a function of distance from significance threshold. Results are displayed on the MNI152 ICBM 2009c non-linear asymmetric average brain. Split stringed violin plots depict observed context-dependent connectivity changes (beta weights) for active and sham tFUS. White dots indicate observed values for sham- (left/red distribution) and active (right/blue distribution), with individual participant observations connected by black lines. Widest lines directly adjacent to white dots indicate the mean value at that time point, with connecting lines above/below indicating the within-subject 95% confidence interval for the mean based upon the Cousineau–Morey method. Asterisks(*) with black connecting lines indicate comparisons that are statistically significant.

Supplementary Figure 5. Changes on Secondary Outcomes from Before to After Repetitive Transcranial Focused Ultrasound

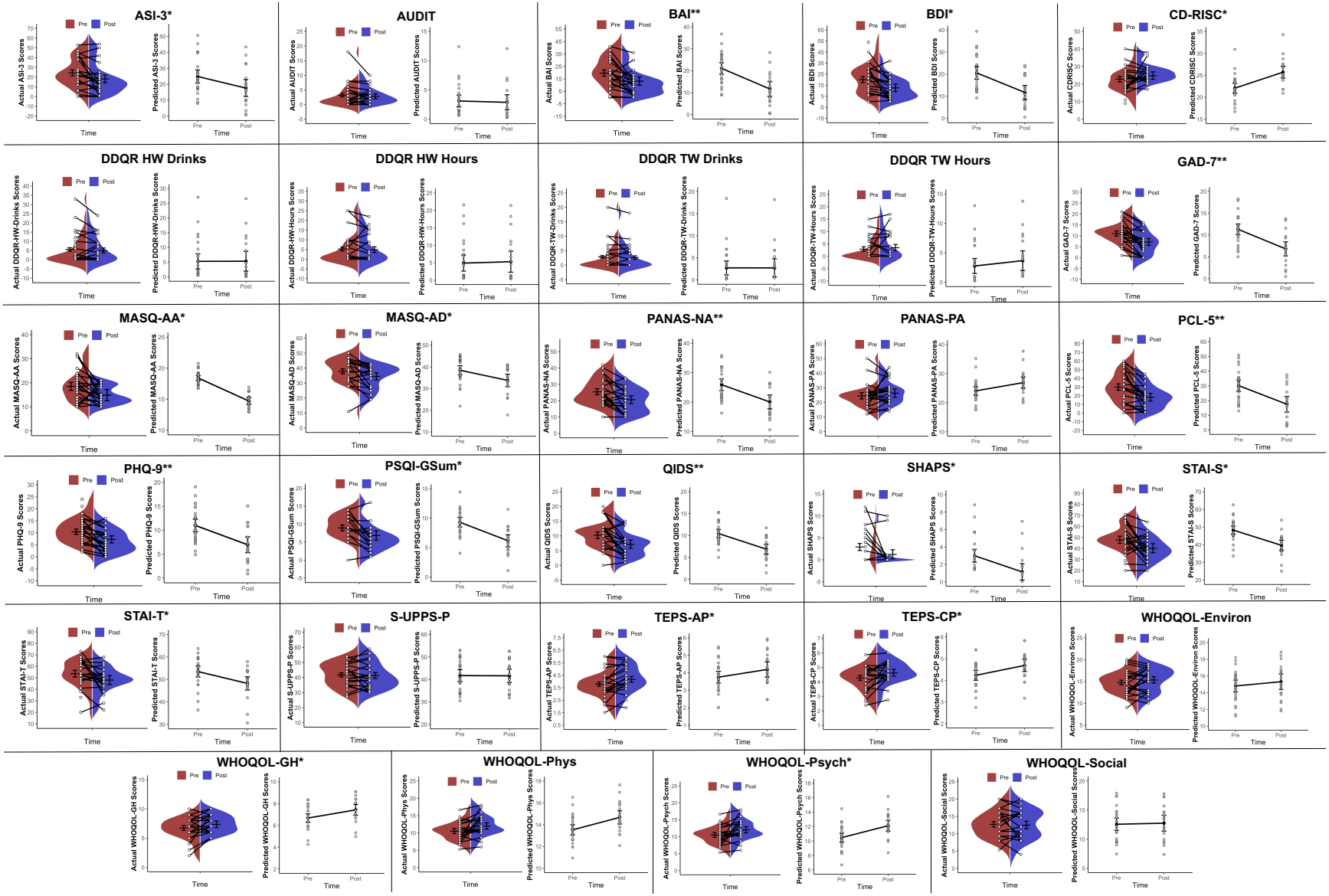


Figure depicts changes on secondary outcomes, with measures showing a significant ($p < 0.05$ uncorrected) change over time noted with an asterisk (*) next to the measure name. Those secondary measures exceeding the Bonferroni-corrected threshold ($p < 0.0017$) for multiple comparisons are marked with a double asterisk (**). For each measure, a split stringed violin plot of observed values is depicted on the left side of each measure section, and line graphs of individual predicted values from the linear mixed effects model analysis are depicted on the right side of each measure section. For split-stringed violin plots, white dots indicate observed values at pre- (left/red distribution) and post-rtFUS (right/blue distribution), with individual participant observations connected by black lines. Widest lines directly adjacent to white dots indicate the mean value at that time point, with connecting lines above/below indicating the within-subject 95% confidence interval for the mean based upon the Cousineau–Morey method. For line graphs, gray dots indicate individual predicted values at pre- and post-rtFUS time points. Black line connects points at the mean of each distribution at each time point. Error bars indicate +/- 1 standard error at each time point. ASI-3=Anxiety Sensitivity Index 3; AUDIT=Alcohol Use Disorders Identification Test; BAI=Beck Anxiety Inventory; BDI=Beck Depression Inventory 2; CD-RISC= Connor-Davidson Resilience Scale; DDQR-HW-Drinks=Number of alcoholic drinks consumed on heaviest week of drinking from Daily Drinking Questionnaire Revised; DDQR-HW-Hours=Number of hours spent consuming alcohol on heaviest week of drinking from Daily Drinking Questionnaire Revised; DDQR-TW-Drinks=Number of alcoholic drinks consumed on a typical week of drinking from Daily Drinking Questionnaire Revised; DDQR-TW-Hours=Number of hours spent consuming alcohol on a typical week of drinking from Daily Drinking Questionnaire Revised; GAD-7=Generalized Anxiety Disorder 7; MASQ-AA=anxious arousal subscale from 30-item Mood and Anxiety Symptom Questionnaire; MASQ-AD=anhedonic depression subscale from 30-item Mood and Anxiety Symptom Questionnaire; PANAS-NA=negative affect subscale from the Positive and Negative Affect Schedule Short Form; PANAS-PA=positive affect subscale from the Positive and Negative Affect Schedule Short Form; PCL-5=PTSD Checklist for DSM-5; PHQ-9=Patient Health Questionnaire 9; PSQI GSum=global summary score from the Pittsburgh Sleep Quality Inventory; QIDS=Quick Inventory of Depressive Symptomatology Self Report; SHAPS=Snaith Hamilton Pleasure Scale; STAI-S=Spielberger State-Trait Anxiety Inventory State Anxiety; STAI-T=Spielberger State-Trait Anxiety Inventory Trait Anxiety; S-UPPS-P= short version of the Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency Impulsive Behavior Scale total score; TEPS-AP=anticipatory pleasure facet of the Temporal Experiences of Pleasure Scale; TEPS-CP= consummatory pleasure facet of the Temporal Experiences of Pleasure Scale; WHOQOL-Envion=environmental health subscale of the WHO Quality of Life Inventory BREF; WHOQOL-GH=general health subscale from the WHO Quality of Life Inventory BREF; WHOQOL-Phys=physical health subscale from the WHO Quality of Life Inventory BREF; WHOQOL-Psych=psychological health subscale from the WHO Quality of Life Inventory BREF; WHOQOL-Social=social health subscale from the WHO Quality of Life Inventory BREF.

Supplementary Figure 6. Whole-Brain Exploratory Analysis Detection of Regions Displaying Task Condition-General Changes in Pre- to Post-Treatment Functional Magnetic Resonance Imaging Activation

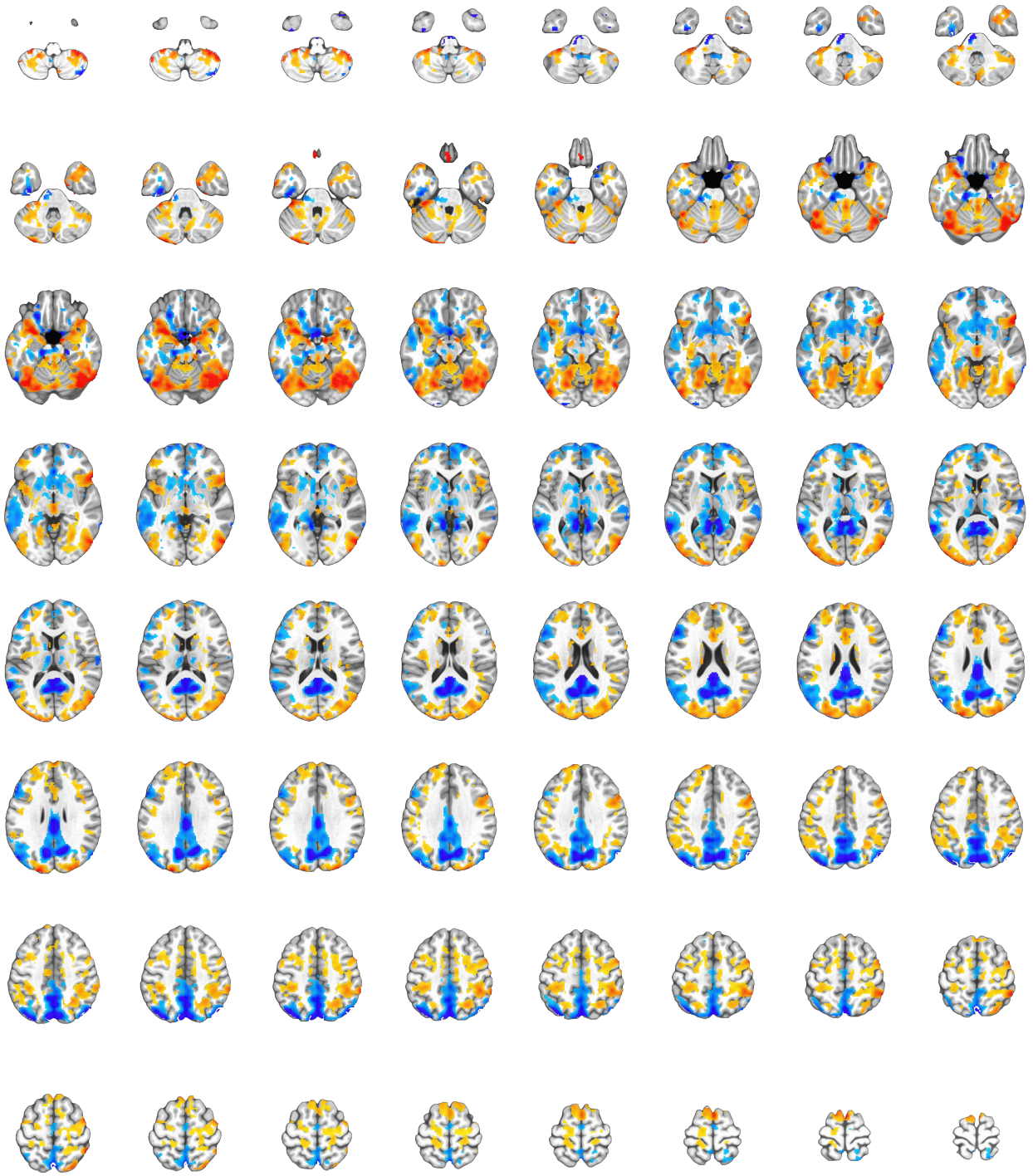


Figure depicts a montage plot of brain areas displaying probabilistic threshold-free cluster enhancement corrected significant effects of time from the whole-brain linear mixed effects model analysis. Areas of significant pre- to post-repetitive transcranial focused ultrasound (rtFUS) changes in task-general activation are depicted as colorized and signed based upon the contrast of pre vs. post-rtFUS mean activation values at each voxel. Therefore, warm (red/yellow/orange) colors indicate regions of significantly attenuated activation post-rtFUS, while cool (light to dark blue) colors indicate regions of significantly elevated activation post-rtFUS. Results are displayed on the MNI152 ICBM 2009c non-linear asymmetric average brain.

Supplementary Figure 7. Association of Improvements on Primary Clinical Outcome with Attenuations in Right Amygdala Condition-General Task-Based Functional Magnetic Resonance Imaging Activation

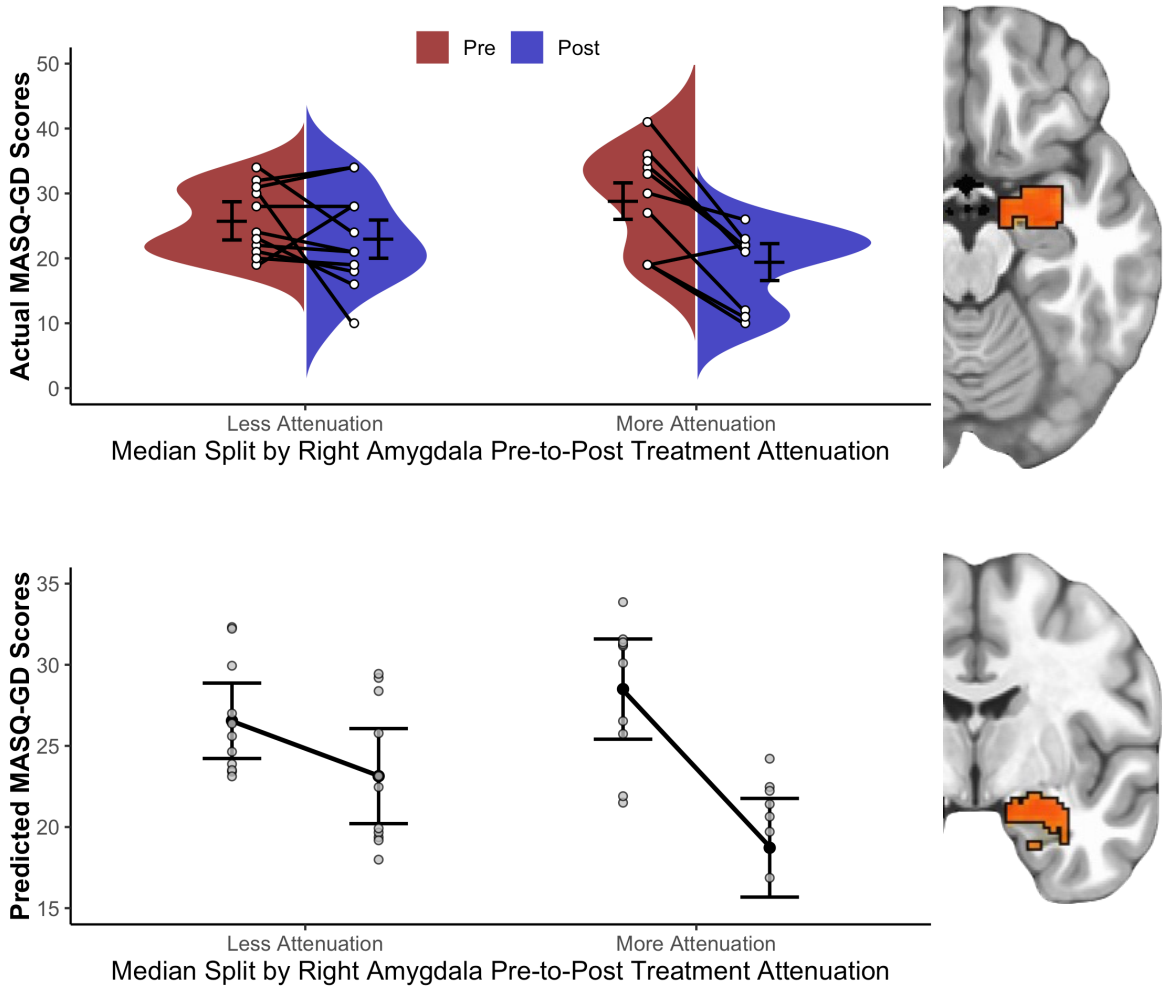


Figure depicts the change in magnitude of individual symptom changes on the Mood and Anxiety Symptom Questionnaire General Distress subscale (MASQ-GD) as a function of average individual attenuations in right amygdala functional magnetic resonance imaging (fMRI) activation from pre- to post-repetitive transcranial focused ultrasound (rtFUS) across all emotional face processing task conditions. Changes in MASQ-GD scores are presented as a median split based upon individual change in right amygdala activation for visualization purposes, but analysis was conducted using continuous variables. Left half of each graph depicts changes in MASQ-GD scores in individuals displaying below-median (less) pre-to post-rtFUS right amygdala activation attenuation, while right half of each graph displays changes in MASQ-GD scores in individuals displaying above-median (greater) pre-to post-rtFUS right amygdala activation attenuation. Top graph displays split stringed violin plots of observed MASQ-GD scores pre- and post-rtFUS. White dots indicate observed values at pre- (left/red distribution) and post-rtFUS (right/blue distribution), with individual participant observations connected by black lines. Widest lines directly adjacent to white dots indicate the mean value at that time point, with connecting lines above/below indicating the within-subject 95% confidence interval for the mean based upon the Cousineau–Morey method. Lower graph depicts the individual predicted values from the linear mixed effects model analysis. Gray dots indicate individual predicted values at pre- and post-rtFUS. Black line connects points at the mean of each distribution at each time point. Error bars indicate +/- 1 standard error at each time point. Brain pictures on right depict the probabilistic threshold-free cluster enhancement-corrected main effect of time within the right amygdala displayed on the MNI152 ICBM 2009c non-linear asymmetric average brain.