TRANSCRANIAL ULTRASOUND AS A POTENTIAL INTERVENTION FOR DEPRESSION

By

Samantha J. Reznik

Copyright © Samantha Jill Reznik 2016

A Thesis Submitted to the Faculty of the
DEPARTMENT OF PSYCHOLOGY
For the Degree of
MASTER OF ARTS
In the Graduate College
THE UNIVERSITY OF ARIZONA
2016
STATEMENT BY AUTHOR

The thesis titled *Transcranial Ultrasound as a Potential Intervention for Depression* prepared by Samantha Reznik has been submitted in partial fulfillment of requirements for a master’s degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Samantha Reznik

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

John J.B. Allen
Distinguished Professor of Psychology

Defense date
5-9-2016
Acknowledgements

Thank you to my mentor John Allen and my committee Mary-Frances O’Connor and Dave Sbarra. Thank you to J. Sanguinetti and the following research assistants who made this work possible: Alyssa Dormer, Michael Lazar, Sara Lomayesva, Alex Schafer, Rohin Singh, Natalia Quintero. Funding from the Graduate Professional Student Council (GPSC) Research and Project Grant made this work possible.
Table of Contents

List of Figures and Tables.............5
Abstract.....................................6
Background.................................7
Method......................................12
Results.....................................21
Discussion.................................30
References.................................35
Tables.......................................47
Figures.....................................49
Appendix........................-----------57
List of Tables

1 Descriptive Statistics .......................47
2 Potential Moderators .......................48

List of Figures

1 Consort Chart .............................49
2 TUS Probe Location .......................51
3 Study Design ..............................52
4 Worry Change ............................53
5 BDI Change by Depression Status ........54
6 Change in Mood ..........................55
7 Change in State Anxiety .................56

List of Appendices

1 Self-Report SCID ..........................57
2 Follow-Up Survey .........................60
Abstract

Anxiety and depression are highly prevalent and often comorbid disorders that cause significant personal and economic burdens (Lépine, 2001). Because a significant number of people with depression do not respond to therapy (Fava, 2003), the development of alternative treatment methods may lessen the burden of such mental disorders. Recent research has focused on brain stimulation methods, many of which require invasive surgery or have limited precision in targeting specific areas. Transcranial ultrasound (TUS) is an alternative, noninvasive brain stimulation method that has greater spatial precision than existing methods (Tufail, 2011). TUS has been found to excite neurons in animal brains (Tufail et. al, 2010) and increase positive mood in humans (Sanguinetti et al, 2013). The present study examined TUS, for the first time, as a potential mood intervention. Twenty-four college students with mild to moderate depressive symptoms were randomly assigned to TUS stimulation or TUS sham (no power administered). Participants completed one TUS session each day for five days. Although depression scores did not change differentially for TUS/Sham, trait worry decreased in the stimulation but not the sham condition. Additionally, those in the stimulation condition rated themselves significantly less tense ten minutes after stimulation compared to those in the sham condition. TUS stimulation did not impact a brain electrical activity index associated with approach motivation, frontal asymmetry. These results have significant implications for the potential utility of TUS as an intervention for anxiety disorders or worry-related psychopathology, warranting future investigation of implicated brain electrical activity and mood changes.

Keywords: TUS, Intervention, Depression, Worry
Transcranial Ultrasound as a Potential Intervention for Depression

Depression affects an estimated 98 million people in the United States, often leading to substantial personal distress and impairment (Mathers, Boerma, & Ma Fat, 2004). Furthermore, up to half of individuals with depression do not respond to traditional antidepressant treatment (Fava, 2003). Because of the debilitating nature of depression and the large number of individuals who experience treatment-resistant depression, alternative treatment options for depression are greatly needed. Brain stimulation presents an alternative, direct treatment approach for depression, including treatment-resistant depression. Yet, current brain stimulation methods have significant limitations. Deep-brain stimulation (DBS) requires surgery, and non-invasive brain stimulation methods such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are limited in their precision and their ability to target specific regions of the brain, particularly deeper structures. Identifying new, noninvasive brain stimulation methods has the potential to improve treatment for depression. Transcranial ultrasound (TUS) remains a less frequently used brain stimulation methodology that has significant advantages over other brain stimulation techniques, as it is both non-invasive and more precise (Tufail, 2011). TUS can excite neurons in animal brains (Tufail et. al, 2010) and has recently been shown to increase positive mood in healthy humans (Sanguinetti, Smith, Dieckman, Vanuk, Hameroff & Allen, 2013).

Despite findings that TUS may increase positive mood in healthy participants, it remains unknown whether TUS can improve mood in individuals experiencing depressive symptoms. The present study examined the effect of a five-session TUS stimulation intervention on subjective mood report, depressive and anxiety symptoms,
and measures of brain electrical activity in individuals with mild to moderate levels of depressive symptoms. The present study represents both the first use of TUS in individuals with depressive symptoms and the first systematic investigation using daily TUS sessions in humans. This double-blind short-term pilot intervention study was aimed at providing insight into the feasibility of using TUS as an intervention for depression and has the potential to lead to an effective, noninvasive brain stimulation treatment for mood disturbance.

**Ultrasound Can Stimulate or Inhibit Tissue**

Ultrasound is a sound wave with a frequency between 20 kHz and 8 MHz, beyond the range of human hearing. Medical professionals commonly use pulsed ultrasound at a frequency of 1 MHz to 15 MHz to image anatomical structures, including fetuses in utero (ter Haar, 2007). Ultrasound is often used at or below 1 MHz for a variety of medical treatments, including physical therapy and cancer ablation (O’Brien, 2007). Ultrasound can be transmitted across bone and soft tissue as pulsed or continuous waves. High-intensity ultrasound can produce heat or cavities (Wu & Nyborg, 2008) and damage cells and tissue, whereas low-intensity ultrasound can stimulate or inhibit muscle and nerve tissues without damage or heat production (Bystritsky et al., 2011). Low-intensity ultrasound has been used safely on biological tissue for therapeutic applications for over 70 years and has been shown to have no lasting bioeffects (ter Haar, 2007). Low-intensity ultrasound has been proposed as a promising therapeutic intervention for neurological and psychiatric conditions because it can excite tissue and target specific areas of the brain. Additionally, both high and low intensity ultrasound can penetrate the skull with great precision (Bystritsky et al., 2011; Colucci, Strichartz, Jolesz, Vykhodtseva, &
Hynynen, 2009).

As early as 1929, Harvey used low-intensity ultrasound to excite nerve and muscle cells in frogs (Harvey, 1929). Fry also found that spontaneous activity in crayfish ventral nerve cords (Fry, Wulff, Tucker & Fry 1950) and electrical potentials over the visual cortex of cats (Fry, 1958) could be temporarily suppressed. Gavrilov (1976) first extended this work to humans by stimulating nerves in the arm and found that ultrasound could induce somatic sensations, such as tactile, thermal, and even pain, without damage (Gavrilov, 1984; Gavrilov et al., 1976). Brain electrical activity has also been suppressed or enhanced in cats and rabbits (Velling & Shklyaruk, 1988). Additionally, ultrasound has been used to examine the relationship between evoked potentials and pain in humans (Wright & Davies, 1989; Wright, Davies & Riddell, 1991) and stimulate the internal ear (Gavrilov, 1984).

Recently, Tsui et. al (2005) found that action potentials in the sciatic nerve of the frog could be inhibited with longer durations of ultrasound pulses and excited with shorter durations of ultrasound pulses. This work suggests that ultrasound pulse duration impacts stimulatory or inhibitory effects of ultrasound (Tsui, Wang, & Huang, 2005). Ultrasound has been used to excite neurons in both hippocampal slices of the mouse brain (Tyler et al., 2008) and in an intact mouse cortex (Tufail et al., 2010) with a spatial precision of around two millimeters. Tufail et. al (2010) found that ultrasound evoked motor movement in mice without changing the motor cortex. Reversible suppression in the sciatic nerve of bullfrogs has also been found for up to 45 minutes (Colucci et al., 2009). Yoo et. al (2011) used real-time functional magnetic resonance imaging (fMRI) concurrently with ultrasound in rabbits. They found that blood oxygenation level
dependent (BOLD) fMRI signal inhibition and activation occurred by varying parameters. Additionally, cortex response to visual stimuli could be reversibly suppressed for over ten minutes. These findings suggest that unlike TMS and other brain stimulation methods, ultrasound may be used to stimulate structures in the brain with a high degree of spatial precision (Yoo et al., 2011).

**Transcranial Ultrasound: A Novel Brain Stimulation Method**

TUS is low-intensity ultrasound that can penetrate the skull. Hameroff et al. (2013) used TUS for the first time in humans. Fifteen seconds of ultrasound was applied at a frequency of 8 Mhz to the fronto-temporal cortex, opposite the site of pain for chronic pain patients. Self-reported pain slightly decreased and self-reported positive mood significantly increased for those in the TUS stimulation compared with the placebo condition at 10 minutes and 40 minutes after stimulation. Ultrasound images verified the ultrasound had penetrated the skull. This first use of TUS in humans confirmed that this novel brain stimulation method can be safely used across the human skull and induce mood changes.

A small number of recent studies have expanded on this work to examine the effect of TUS on self-reported mood in healthy participants. Sanguinetti et al. (2013) found that TUS at 2 MHz significantly increased positive mood compared to TUS at 8 MHz for 15 seconds of TUS stimulation at the right fronto-temporal area. Thirty seconds of TUS stimulation at this optimal frequency increased positive mood significantly at both 15 and 30 minutes after stimulation; a sham condition produced no mood effects. These findings suggest that lower frequency (2 compared to 8 MHz) and longer stimulation (30 compared to 15 seconds) may be better for penetrating the skull for
neuromodulation. In a subsequent and larger study of 147 healthy participants, Sanguinetti et al., (2014) found that 30 seconds of TUS stimulation at a frequency of 0.5 MHz with a pulse repetition frequency of 40 Hz stimulation at the right fronto-temporal cortex increased positive mood. These findings suggest that TUS at the right fronto-temporal cortex may be used to increase mood in human participants.

The Potential Impact of TUS on Electrical Activity

Velling & Shklyaruk (1988) found that ultrasound can inhibit or excite brain electrical activity in cats and rabbits. Yet, changes in brain electrical activity due to TUS in humans remain largely unexplored. As such, a brain electrical activity index (frontal asymmetry) linked with approach systems was investigated in response to TUS. Changes in frontal asymmetry in response to TUS would represent meaningful change as these indices have been linked with approach motivation, a system in which disturbances may indicate vulnerability to depression (Allen & Reznik, 2015).

Frontal asymmetry is a relative measure of the difference in electroencephalogram (EEG) alpha power between the right and left frontal regions. As alpha activity has an inhibitory influence on cortical network activity, lower frontal asymmetry scores (right minus left alpha) putatively reflect relatively less left than right cortical activity (Allen, Coan, & Nazarian, 2004). Less relative left frontal activity during rest has been associated with decreased approach motivation. The approach- withdrawal motivational model of frontal asymmetry proposes that left-sided frontal regions are primarily involved with approach-related affect such as elation, hope, happiness, and anger (Depue & Iacono, 1989; Eddie Harmon-Jones, 2003). On the other hand, right-sided prefrontal regions are associated with withdrawal such as behavioral inhibition and vigilant
attention that often complement certain negative affective states (Coan & Allen, 2004; Davidson & Irwin, 1999; Harmon-Jones & Allen, 1998). The approach system regulates goal-directed behavior and appetitive motivation and responds to cues for reward and for punishment avoidance. The withdrawal system is sensitive to stimuli associated with punishment and the termination of reward (Davidson & Irwin, 1999).

**Present Study**

The present study examined the effect of a one-week TUS intervention on mood and anxiety symptoms. Because prior research has found that the right fronto-temporal cortex is implicated in mood (Habel, Klein, Kellermann, Shah, & Schneider, 2005) and that TUS in this region increases mood (Sanguinetti et al., 2014), stimulation occurred at the right fronto-temporal cortex. Participants were randomly assigned to a TUS stimulation or TUS sham (no power) condition. They completed five sessions of TUS stimulation or sham within seven days. Depressive symptoms, impairment due to anxiety, self-reported mood, and frontal asymmetry were examined each day. Given that TUS has been found to improve mood in humans (Sanguinetti et al., 2014), we predicted that participants exposed to the TUS stimulation condition would have reduced depressive symptoms compared to participants exposed to the sham condition. Additionally, we expected increased relative frontal activity after stimulation, consistent with increased approach motivation. Results in line with prediction would have important implications for the potential utility of TUS as an intervention for depression, warranting future work examining TUS as an alternative depression treatment.

**Method**

**Participants**
Twenty-four (16 women, 8 men) participants with mild to moderate depression completed the study (M=18.92, SD =1.10). Participants who scored between 10 and 25 on the Beck Depression Inventory-II (BDI-II) were recruited from the Introductory Psychology pool at the University of Arizona (see Figure 1). Participants were right-handed, fluent English speakers and had no serious medical conditions, head injury, or severe headaches. Additionally, participants taking psychotropic medication or seeking other treatment as well those with current active suicidal potential necessitating immediate treatment were excluded and referred to mental health services. Participants provided informed consent before participation, and the University of Arizona Institutional Review Board approved the protocol.

Procedure

Introductory Psychology Students who scored between 9 and 27 on the BDI-II during a large survey at the beginning of the semester were invited to complete an online screener. Those eligible to complete the study were contacted via telephone. Eligible and interested participants scheduled five laboratory visits. Those who did not qualify for the study or qualified for the study but chose not to participate were provided with a list of mental health resources in the community via e-mail (see Figure 1 for Consort Chart). Participants were randomly assigned to either TUS stimulation or TUS sham (no power) before their first visit. Participants were matched between conditions by age, sex, and level of depressive symptoms. Participants were invited to participate in five laboratory visits within a seven-day span where they would receive TUS stimulation/sham at the right fronto-temporal area. At the end of five days, participants were invited to continue for another five days at their discretion. Only data from the first five days are reported.
here, as only 3 participants completed the second five sessions. Participants were reminded that they could withdraw at any time.

At each laboratory visit, participants completed a number of state and trait self-report questionnaires as well as receiving TUS stimulation/sham. Additionally, EEG was recorded during the first visit and during the fifth visit. In the sham condition, the ultrasound probe was placed at the right fronto-temporal area without stimulation. As noted above, prior research has shown that stimulation at this area induces positive mood change and is implicated in positive mood (Habel et al., 2005, see Figure 2 for placement of probe). All research assistants and participants remained blind to the condition assignment of participants.

Following consent at the first visit, participants again completed a survey of exclusionary criteria and a modified version of the credibility/expectancy scale (Devilly & Borkovec, 2000). All mentions of trauma symptoms in this scale were replaced with “mood symptoms”. Participants also completed a paper-and-pencil self-report version of the Structured Clinical Interview for the DSM-5 (SCID-5) in which they marked every symptom of depression they currently experience, number of symptoms they experienced during their worst period of depression, and the number of times they have felt depressed (see Appendix 1).

On visit one and visit five, after EEG cap application, participants completed six minutes of resting baseline EEG recordings. As in prior research, the resting period consisted of a counterbalanced sequence of minute-long eyes-open and eyes-closed segments (Tomarken, Davidson, Wheeler, & Doss, 1992). At the end of the laboratory visit, participants completed another six minutes of resting baseline EEG recordings to
examine changes in brain electrical activity over the course of the session.

Research assistants identified the right fronto-temporal area using electrode site F8 and placed the ultrasound probe at this area. Both research assistants remained in the room with the participant for the administration period of 30 seconds. After this period, participants were asked to rest for ten minutes without a task and then twenty minutes without a task. Participants were monitored via video camera from a room next door as they rested. If participants appeared to be tired to the point of falling asleep, research assistants interrupted the participant to let them know how long they had left. Otherwise, participants and research assistants only interacted 10 and 20 minutes post the TUS administration in order to administer the mood assessments.

**Self-report Assessments.** State mood was assessed three times at each of the sessions: before TUS stimulation, 10 minutes after TUS stimulation, and 30 minutes after TUS stimulation. These three time points allowed for assessment of the acute effects of TUS stimulation as well as repeated TUS stimulation over days on self-reported mood. The Visual Analogue Mood Scales (VAMS; Arruda, Stern, & Somerville, 1999) measured state mood and provided standardized measures of both self-reported Global Affect, which represents happiness/sadness, and self-reported Global Vigor, which represents anxiety and fatigue.

Additionally, depressive symptoms and impairment due to anxiety were assessed after state mood at the end of each session. The Beck Depression Inventory-II (BDI-II; Beck, Steer, & Carbin, 1988) is a widely-used measure of depressive symptoms that allows for an overall impression of severity and spans both cognitive and affective aspects of depression. Additionally, a shorter anxiety assessment, called the Overall
Anxiety Severity and Impairment Scale (OASIS) was administered each day to track overall severity of anxiety (Norman, Cissell, Means-Christensen, & Stein, 2006).

Given the importance of repetitive thought in maintaining psychopathology, both worry and rumination were examined (Huffziger, Ebner-Priemer, Koudela, Reinhard, & Kuehner, 2012; Treynor, Gonzalez, & Nolen-Hoeksema, 2003). Worry is future-focused repetitive thought typically associated with anxiety disorders such as generalized anxiety disorder, whereas rumination is past-focused repetitive thought typically associated with maintenance and onset of depression. Trait rumination was assessed using the Ruminative Responses Scale (RRS, Nolen-hoeksema, Wisco, & Lyubomirsky, 2014) and worry was assessed using the Penn State Worry Questionnaire (PSWQ, Brown, Antony, & Barlow, 1992). In order to limit the number of questions participants must respond to each day, the RRS and PSWQ were only administered on the first and the fifth days. Each day, a 2-item state rumination assessment by Moberly and Watkins (Moberly & Watkins, 2008) was included before and after TUS stimulation. Participants were asked to report how much they are considering their problems and feelings at the present moment. These two questions were answered prior to the mood assessment, given the potential of the mood assessment to create ceiling effects when participants evaluate how much they are considering their feelings.

Days two through four followed the same protocol but excluded the EEG recordings in order to reduce participant burden. Day 5 followed the same protocol and participants were provided the option to continue the study for an additional five sessions. If participants chose to continue, the procedure remained the same, and EEG was recorded Day 10. In order to keep the paradigm as similar as possible between days with and
without EEG data collection, resting data were collected after mood and depressive symptoms questionnaires on days with EEG collection (see Figure 3 for structure of sessions). Participants were compensated for their time.

One month after participants completed the study, they were contacted for an online follow-up. They completed an OASIS and BDI-II online as well as answering a number of questions about whether or not they enjoyed the study or would recommend the study to a friend (See Appendix 2). BDI Item 21 (Loss of interest in sex) was unintentionally omitted from the one-month follow-up. All analyses using the follow-up BDI were compared only to altered BDI measures omitting Item 21.

**Ultrasound Parameters.** Ultrasound stimulation occurred using the Neurotrek U+™, an ultrasound device developed by Thync Inc., Los Gatos, CA. The Food and Drug Administration (FDA) recommends acoustic output below 720 mw per square centimeter, a mechanical index of 1.9, and a thermal index of 6.0 to avoid heating. Thermal index and mechanical index were both estimated, and maximum acoustic output was well below the FDA limit (Food and Drug Administration, 1999).

The U+™ device was set at a power of 11%, a frequency of 0.5 MHz, and duration of exposure of 30s. Prior research has used the same parameters with power at 21% (Sanguinetti et al., 2014). Lower power was utilized in this study in order to use conservative amounts of energy due to the repeated stimulations in this study. Additionally, the U+ device was utilized on “experimenter” mode. In this mode, researchers may input a sequence of five numbers that turns the ultrasound to stimulation or sham (unknown to researcher and participant). A PhD-level scientist not directly affiliated with recruitment or subject running had access to all conditions of the
participants and gave researchers daily 5-digit codes. As such, all researchers who interacted with participants had no knowledge of their condition.

**EEG Recording and Reduction.** EEG data were collected using a 64-channel electrode cap and NeuroScan Synamps2 amplifier. Four electrode leads were placed on the inferior and superior orbit of the left eye and on the outer canthi to record blinks and horizontal movements. Impedances for all sites were kept at or below five KΩ throughout the session. Signals were bandpassed online from 0 to 200 Hz and amplified with a gain of 2816 and sampled at 1000 Hz. Signals were recorded using an online reference site immediately posterior to Cz and converted to a reference-free CSD derivation offline.

After acquisition, continuous data using the online reference was visually inspected, and trained laboratory assistants rejected epochs with muscle movement or other artifact. Data files were epoched into 2.048 second segments. The two ocular channels and mastoids were excluded for a total of 60 scalp sites after transformation. Epoched data were processed using Independent Component Analysis (ICA) to identify and remove blinks, ocular artifacts, and signal discontinuities using the ADJUST algorithm (Mognon, Jovicich, Bruzzone, & Buiatti, 2011). An artifact-rejection algorithm was then applied to remove any possible remaining spikes and DC shifts. Data were converted to the reference-free CSD transformation via the CSD Toolbox of Kayser and Tenke (Kayser & Tenke, 2006). Epochs were overlapped at 75% and a Fast Fourier Transform (FFT) was applied to the artifact free epochs to obtain power spectra, following weighting using a Hamming window to prevent spurious estimates of spectral power. Power spectra across all epochs without artifact were averaged.

Total alpha power (8-13 Hz) was extracted from each site, as in prior frontal
asymmetry research (Tomarken, Davidson, Wheeler, & Kinney, 1992). The asymmetry index was computed as the difference in the natural log of a sensor on the right hemisphere and the natural log of its homologous sensor on the left hemisphere [ln(right) minus ln(left) alpha power]. Lower frontal asymmetry scores putatively reflect relatively less left than right cortical activity (i.e. relatively more left alpha, as alpha has an inhibitory influence on cortical network activity (Towers & Allen, 2009). As less relative left frontal activity at rest has been associated with depression (Schaffer, Davidson, & Saron, 1983), an increase in relative left frontal activity was predicted if TUS increased mood.

Data Analysis

Symptom Outcome Variables. Due to the small number of participants in this study, within-person effect sizes were of primary interest. First, the effect of condition on change in all major symptom outcome variables (BDI Change, OASIS Change, PSWQ Change, and RRS Change) was examined. A difference score between Day 5 and Day 1 was computed for each major symptom outcome variable. A correlation, which represents an effect size, was conducted between condition (Sham/Stimulation) and all of these major difference scores. As difference scores eliminate a large amount of the variance in daily depressive symptom recordings, individual regressions were used to examine slopes of change in measures that were collected each day (BDI, OASIS). An individual regression was calculated for each participant. A correlation was again used to examine the effect of condition (Sham/Stimulation) on slope. As such, this analysis allows for an estimate of the size of effect of the Condition on rate of change in participants.
**Moderation by Individual Differences.** Additionally, exploratory analysis of individual differences was conducted, examining the correlation between change in outcome variables and Condition in different groups. Then, nondependent samples differences in correlations were computed. Those relationships with a Z-score over one were explicated in the text below. First, history of major depression was examined. Based on the self-report SCID, those who met clinically significant criteria for one or more current or past depressive episodes (Previously Depressed) were compared to those who did not meet clinically significant criteria (Never Depressed). This comparison essentially compares more clinically severe to less clinically severe cases of individuals experiencing mild to moderate depressive symptoms. Additionally, participants were split at the mean of the RRS to compare High and Low Rumination participants and on the PSWQ to compare High and Low Worry participants. In addition to examining independent correlations of each group, a 2 (Condition) by 2 (Group) by 2 (Time) mixed-design analysis of variance (ANOVA) examined differential change as a function of treatment condition and individual differences. Condition was TUS Sham or Stimulation, Group was a binary variable in which individuals were marked as Previously Depressed or Never Depressed, High or Low Rumination, or High or Low Worry participants. Time included Day 1 and Day 5. Outcomes were all major outcome variables.

**Within-Day Mood & Rumination Effects.** In order to replicate the analysis of prior studies (Sanguinetti et al., 2014), a 2 (Condition) by 3 (Time) mixed-design analysis of variance (ANOVA) was conducted to examine the effect of condition on mood. Condition, a between-subjects factor, represents participants in either the TUS stimulation or TUS sham. Time, a within-subjects factor, involved a measurement at
three time points: before TUS administration, 10 minutes after TUS administration, and 30 minutes after TUS administration. This time structure allowed analysis of the slope of mood before and after the ultrasound. All days were included and day was included as a covariate, but not the interaction of day and other factors. Effect sizes here are reported as the proportion of variance accounted for ($\eta_p^2$). Additionally, in order to examine how the effect size of condition may change over time, correlations between Condition (Sham/Stimulation) and difference score between initial rumination and rumination 10 minutes out as well as difference score between initial rumination and 30 minutes out were examined. This analysis was conducted for the two standardized mood measures commonly used from the VAMS. Global Affect represents the amount of positive/negative affect an individual currently reports, whereas Global Vigor indicates the amount of emotional arousal that they currently report. Additionally, this analysis was repeated for state rumination also collected at the same time points. All of these analyses were repeated for Day 1 only in order to replicate prior research of only one day.

Within-Day Brain Electrical Activity Effects. A 2 (Condition) by 2 (Time) mixed-design analysis of variance (ANOVA) was used to examine the effect of TUS stimulation or sham on frontal asymmetry. Condition again represented participants in either the TUS stimulation or TUS sham and Time was resting EEG at the beginning of the session and end of the session. Day was included as a covariate. Effect sizes were again examined as $\eta_p^2$.

Results

Descriptive Statistics
Twenty-six participants began the study. Participants were matched to treatment group by age, sex, and BDI-level. Participants were recruited such that there were at least eight individuals (four pairs) in each BDI level (10-15, 16-20, and 21-25). Random assignment ensured that both conditions had participants with the same depression levels, age, and gender but also that individuals from the full range of symptoms in this study (10-25) were represented evenly. Fourteen participants were assigned to the Sham Condition and 12 participants were assigned to the Stimulation Condition. Two participants from the Sham Condition were excluded: one due to only 1 day of data and the other due to wide variation in BDI scores corresponding to meaningful life events during the course of the study. Participants were excluded by consensus of the PI and supervisor, blind to condition of the participant. Two additional participants were included to replace these two in the Sham Condition at the end of the study (condition unknown to experimenters and participants). Of the 24 participants included in the following analyses (12 Sham, 12 Stimulation), 22 completed the study until at least Day 5. Two participants (both in Sham Condition) completed the study until Day 4. Three participants went beyond Day 5: one until Day 7 (Sham Condition), one until Day 8 (Stimulation Condition), and one until Day 10 (Sham Condition). Figure 1 is the consort chart for the present study. All days were included in analyses where relevant. There were no differences between Stimulation and Sham Condition on BDI or OASIS. However, there was a difference on both the PSWQ ($t=2.194$, $p=.039$) and RRS ($t=2.532$, $p=.019$), representing repetitive thought (see Table 1). Thus any significant differences between groups were rerun with level of repetitive thought as a covariate to determine if this difference could account for the effects.
**Symptom Outcome Variables**

The effect of Condition on BDI Change was examined using a difference score in depression level from Day 5 to Day 1. Contrary to expected results, there was no effect of Condition, and descriptively those in the Sham Condition had slightly larger decreases in depression scores than those in the Stimulation Condition, $r = -.237$, $p = .288$. This finding suggests that the present study did not find that TUS stimulation positively impacted depressive symptoms. There was no effect of Condition on individual slopes, $r = .06$, $p = .782$.

This analysis was repeated for the OASIS Change in order to examine impairment related to anxiety over the course of the study. A difference score on the OASIS from Day 5 to Day 1 was calculated. Again, contrary to expected results, there was a small effect of Condition such that those in the Sham Condition had slightly larger decreases in anxiety scores than those in the Stimulation Condition, $r(22) = -.235$, $p = .293$. Again, individual regressions were used to examine the slope of change in each participant. There was no effect of Condition on individual slopes, $r(22) = .032$, $p = .885$. This finding suggests that overall impairment due to anxiety, as measured by the OASIS, was not impacted by five days of TUS stimulation.

Additionally, the potential impact of the intervention on repetitive thought associated with psychopathology was assessed using the RRS, a measure of trait rumination, and PSWQ, a measure of trait worry. There was no effect of Condition on RRS Change from Day 5 to Day 1, $r(22) = -.024$, $p = .916$. There was a medium effect of Condition on PSWQ Change from Day 5 to Day 1 in the expected direction, $r(22) = .363$, $p = .097$; those in the Stimulation Condition had decreased levels of worry at Day 5.
compared to Day 1 (M=-1.83, SD=7.73), whereas those in the Sham Condition had increased levels of worry at Day 5 compared to Day 1 (M=3.30, SD=5.67). When adjusting for level of PSWQ at the first session using a partial correlation, the effect was slightly weakened but still showed more change in Worry in the Stimulation Condition compared to Sham Condition, r=.277, p=.244. When adjusting for level of rumination at the first session using a partial correlation, the effect was slightly strengthened, r=.382, p=.087. These findings suggest that the Stimulation Condition decreased worry compared to the Sham Condition (see Figure 4), even after accounting for initial repetitive thought. As these measures were only administered on Day 1 and 5, slope analysis was not possible.

**Moderation by Individual Differences**

The full set of moderation analyses are summarized in Table 2. The correlation between change in outcome variables and Condition by different groups was examined. Then, nondependent samples difference in correlation was computed. Those relationships with a Z-score over one were explicated in the text below.

*Moderation by Individual Differences: Depression Status.* In order to examine potential impact of depression-history status, effect-size estimates (correlations) were examined separately for Previously Depressed and Never Depressed participants for all major outcome variables. Potential moderators will be discussed here but Table 2 shows all potential moderation analyses conducted. Prior and current depression status was determined by using the self-report SCID. The 2 (History Status) by 2 (Condition) ANOVA on BDI Change revealed a positive main effect of History status, F=5.711, p=.028, $\eta_p^2=.241$, and a small effect size for the interaction of Condition by History
Status, $F=1.189$, $p=.290$, $\eta^2_p=.062$ (see figure 5). Decomposing the interaction, for Previously Depressed Participants, Stimulation Condition produced moderately smaller decreases in depression symptoms than Sham Condition ($r=-.523$, $p=.121$) than was the case among Never Depressed participants ($r=.115$, $p=.722$).

The 2 (History Status) by 2 (Condition) ANOVA on RRS Change revealed a positive main effect of Depression status, $F(1,18)=8.287$, $p=.010$, $\eta^2_p=.315$ and a small effect size for the interaction of Depression Status and Condition on RRS, $F=.921$, $p=.350$, $\eta^2_p=.049$. Decomposing the interaction, for Previously Depressed Participants, stimulation produced moderately greater decreases in rumination than Sham Condition ($r=.419$, $p=.228$) than was the case among Never Depressed participants ($r=-.500$, $p=.098$). Overall, for Never Depressed participants, the Stimulation Condition reduces depressive symptoms more than the Sham Condition but does not reduce rumination. For Previously Depressed Participants, the Stimulation Condition reduces rumination symptoms more than the Sham Condition, but did not reduce depressive symptoms.

*Moderation by Individual Differences: BDI Level.* These same relationships held when participants were split into High BDI (above the mean) and Low BDI (at or below the mean) groups from their intake scores, which is not surprising given that the Previously Depressed group had significantly higher BDI levels ($M=19.91$, $SD=3.30$) than the Never Depressed Group ($M=14.85$, $SD=4.259$), $t(22)=-3.198$, $p=.004$.

*Moderation by Individual Differences: High and Low Rumination.* Differential effects for High Rumination versus Low Rumination participants (split at mean) were also examined. The 2 (High Rumination/Low Rumination) by 2 (Condition) ANOVA on BDI Change revealed a positive main effect of High/Low Rumination Status,
F(1,18)=2.457, p=.134, $\eta_p^2=.120$ and a small effect size for the interaction of Depression Status and Condition on RRS, F(1,20)=3.054, p=.096, $\eta_p^2=.132$. Decomposing the interaction, for High Rumination participants, Stimulation did not produce greater change in depression scores ($r=-.683$, p=.020) but Stimulation did produce greater change in depression scores for Low Rumination participants ($r=.467$, p=.147).

The 2 (High Rumination/Low Rumination) by 2 (Condition) ANOVA on RRS Change revealed a positive, significant main effect of High/Low Rumination Status, F(1, 18)=39.633, p<.01, $\eta_p^2=.688$ and a small effect size for the interaction of Depression Status and Condition on RRS, F(1, 18)=1.449, p=.244, $\eta_p^2=.075$. Decomposing the interaction, for High Rumination participants, Stimulation produced more change in Rumination than Sham ($r=.524$, p=.098) whereas for Low Rumination participants Stimulation versus Sham did not cause more change in Rumination ($-.165$, p=.629). Overall, High Rumination participants had a greater change in Rumination scores due to Stimulation than Sham condition but not in depressive symptoms. Low Rumination participants had a greater change in depression scores due to Stimulation rather than Sham Condition, but they did not have a change in Rumination scores.

*Moderation by Individual Differences: High and Low Worry.* Finally, there were no meaningful differences in the 2 (High Worry/Low Worry) by 2 (Condition) ANOVA on all major outcome variables (see Table 2 for summary of correlations).

*One-Month Symptom Follow-Up.* For all participants who completed the follow-up survey one month later, a difference score between BDI and OASIS at follow-up and BDI and OASIS at Day 5 was computed. The effect of Condition on this difference was examined as a correlation. Additionally, the four questions assessing participant
enjoyment and self-experienced mood change were examined as a function of Condition using a correlation. There was no evidence of an effect of Condition on BDI Change at Day 5 to BDI at Follow-up, r(13)=.032, p=.917. Due to accidental omit of Item 21 on BDI at follow-up, BDI Day 5 without Item 21 was used for this analysis. There was a medium effect of Condition on OASIS Change at Day 5 to OASIS at Follow-up, r(13)=.262, p=.387. Those in the Stimulation Condition had decreased impairment due to anxiety (M=-0.8571, SD=4.34), whereas those in the Sham Condition had on average increased anxiety one-month after the end of the study (M=1.00, SD=2.76). There was also a medium and statistically significant effect of Condition on participant-rated enjoyment in the study r=-.556, p=.05. Those in the Stimulation Condition rated the study as significantly more enjoyable (M=4.29, SD=.488) than those in the Sham Condition (M=3.67, SD=5.16). There were small effects of feeling that mood improved from the study, r=-.150, p=.624 and feeling that uncontrollable, negative thoughts improved from the study, r=-.150, p=.185, such that those in the Stimulation Condition rated more positive change than those in the Sham Condition. There was also a small effect of Condition on whether participants would recommend the treatment to other people, r=.135, p=.660, such that those in the Sham condition were more likely to recommend the treatment to someone else than in the Stimulation Condition.

**Within-Day Mood & Rumination Effects**

Global Affect is a measure of self-reported positive and negative affect. There was no main effect of Condition on Global Affect across days, F(1, 108)=.253, p=.623 and effect size of Condition was negligible, $\eta^2_p=.002$ (see Figure 6). Correlations between Condition and Global Affect Change 10 minutes after TUS administration, r(112)=-.130
p=.172, and 30 minutes after TUS administration, r(111)=-.031, p=.749 indicate no effect of Condition on Global Affect across days. To provide a comparison to the findings of Sanguinetti et al. (2013), who assessed the impact of TUS on a single day, an analysis including only Day 1 was conducted. As expected, there was again no main effect of Condition across all three time points, F(1,22)=.470, p=.500, η_p^2=.021, but in line with predictions there was a small interaction between Time and Condition on Global Affect, F(2,22)=2.483, p=.095, η_p^2=101. Correlations between Condition and Global Affect Change 10 minutes after TUS administration, r(24)=-.429, p=.036 indicate a medium effect of Condition on Global Affect Change 10 minutes after TUS administration, such that those in the Stimulation Condition had significantly increased Global Affect compared to those in the Sham Condition (see Figure 7). There was no effect of Condition on Global Affect Change 30 Minutes after TUS administration, r(24)=-.030, p=.890.

Global Vigor is a measure of self-reported emotional arousal. There was a small effect of Condition on Global Vigor across days, F(1, 108)=3.200, p=.076, η_p^2=.029, such that those in the Stimulation Condition had more decreased Global Vigor than those in the Sham Condition. There was a small to negligible effect of the interaction between Condition and Time on Global Vigor across all days, F=2.410, p=.092, η_p^2=.020. Correlations between Condition and Global Vigor Change 10 minutes, r=.113, p=.237 and 30 minutes, r=.173, p=.069 after TUS administration represent small effect sizes in the direction of decreased Global Vigor in those in the Stimulation Condition compared to Sham Condition.
To provide a comparison to Sanguinetti et al. (2013), analyses including only Day 1 revealed, as expected, no main effect of Condition on Global Vigor at 10 and 30 minutes after TUS administration, $F(1,22) = .899, p = .353, \eta_p^2 = .039$. There was a small interaction of Condition and Time on Global Vigor, $F(1,22) = 1.772, p = 1.82, \eta_p^2 = .075$. In analysis of Day 1 only, there was a medium effect of Condition on change in Global Vigor Change 10 minutes after TUS administration, $r(24) = .447, p = .029$, such that those in the Stimulation Condition compared to the Sham condition had significantly decreased Global Vigor, which was later manifest as a medium effect of Condition on Global Vigor Change 30 minutes after TUS administration, $r(24) = .267, p = .207$.

**Within-Day Brain Electrical Activity Effects**

The 2 (Condition) by 3 (Time) mixed-design analysis of variance (ANOVA) to examine the effect of Condition on frontal asymmetry with day (Days 1-5) and site (sites F8/F7, F6/F5, F4/F3, and F2/F1) as covariates was conducted. The between-subjects variable Condition included Sham or Stimulation. The within-subjects variable Time included brain activity before and after TUS stimulation. Day and site were included as covariates. It was found that there was a small effect of Condition, such that those in the Sham Condition actually increased frontal asymmetry from resting before ultrasound to resting after ultrasound more than the Stimulation Condition but this explained only a small amount of variance, $F(1,161) = 3.605, p = .059, \eta_p^2 = .022$.

**Follow-Up Analysis**

It was not expected to see more change in Global Vigor in the Stimulation Condition compared to Sham Condition. The Global Vigor scale includes a number of items related to anxiety (calm, tense) as well as fatigue (alert, weary). As such, follow-up
analysis examined whether the decrease in Global Vigor that occurred in Stimulation Condition may be due to decrease in State Anxiety. A State Anxiety variable was computed including only calm and tense items from the VAMS. Across all days, there was no main effect of Stimulation Condition on State Anxiety, F=.059, p=.808, η²=.002. There was a small statistically significant effect revealing greater decreases among those receiving TUS compared to Sham in State Anxiety from baseline to 10 minutes after stimulation, r(112)=.192, p=.042 (see figure 7) and no meaningful effect 30 minutes after stimulation, r(111)=.022, p=.822.

**Discussion**

The present study found that TUS may decrease state anxiety for up to 10 minutes and improve worry over the course of a short-term intervention. These findings have significant implications for the potential development of a novel, effective, portable, and low-cost intervention for anxiety and worry-related disorders, such as generalized anxiety disorder (GAD). With a prevalence of 3.1% and 1% suffering from impairment due to the disorder in the United States (Kessler, Chiu, Demler, & Walters, 2005), novel and cost-effective treatments for GAD may lessen the personal and economic burden caused by the disorder.

Prior research found that a single dose of TUS can increase positive mood in humans up to 30 minutes later (Sanguinetti et al., 2014). We utilized the same device from this prior research. In prior research, the U+™ device was set at a power of 21%, a frequency of 0.5 MHz, and duration of exposure of 30s. In the present study, we opted for caution and lowered the power to 11% because of the repeated stimulations (five to ten). Given the strength of prior mood effects, we believed that this more conservative
approach would ensure participant safety (given absence of research on repeated stimulation) and still allow for mood effects. However, while this prior research found increased positive mood 30 minutes after TUS, we found increased positive mood only 10 minutes after TUS. It is possible that the decreased power used in this study was responsible for the short-lived mood effects. The prior study also only included healthy participants. As the present study included participants with mild to moderate depressive symptoms, it is also possible that mood effects may differ between healthy and depressed populations. As such, future research will need to explore the potential optimal parameters, considering both safety and efficacy, for a TUS intervention as well as differential population effects. The restricted range of depressive symptoms in the present study allowed for a homogeneous population for a pilot study. Future large-scale research may include a range of psychopathology symptoms, from non-existent to severe to examine how TUS parameters may impact mood in different populations.

Contrary to prediction, TUS did not improve depressive symptoms over the course of the intervention. However, TUS improved worry symptoms. The potential for TUS to decrease worry is clinically relevant as worry is a core feature of GAD, a prevalent disorder that more than 5% of adults will experience in their lifetime (Kessler et al., 2005). Additionally, TUS stimulation decreases Global Vigor at 10 minutes, an effect that appeared to be driven by the anxiety-related items on the Global Vigor scale. Aside from the present study, to date, no research has examined state changes in anxiety due to TUS. These findings are consistent with prior mood findings in that anxiety is most commonly appraised as a negative emotional state and may influence an individual’s overall self-reported mood. However, future research examining the impact of TUS on
state anxiety using an empirically validated state anxiety scale would greatly improve our understanding of whether TUS may be used as an intervention for anxiety disorders.

At the one-month follow-up it was also found that there was no change from study exit in depression scores based on Stimulation Condition but there was change in impairment due to anxiety. Those in the sham condition reported increased impairment due to anxiety since their last day in the study, whereas those in the stimulation condition reported decreased impairment due to anxiety since the last day of the study. Additionally, those in the stimulation condition reported enjoying the study more but were less likely to recommend it to others. Although there was difficulty with retention at the one-month follow-up, these results suggest that the decrease in worry seen during the intervention may have impactful effects on one’s perception of impairment due to anxiety up to a month later. Future research should include one month and longer follow-ups to examine the potential sustained effects of a TUS intervention.

Additionally, the results suggest several potential moderators of the impact of TUS on change in symptoms. We selected several potential moderators, including: depression history status (history positive and history negative as documented in a self-report SCID), low versus high ruminators, and low versus high worriers. These results suggest overall that for individuals with less severe cases of depression (never met clinical criteria for depression or low ruminator groups) TUS may decrease depressive symptoms. Yet for these same groups, TUS does not seem to decrease rumination symptoms. For more severe depression groups (met clinical criteria for depression at least once or high ruminator group), TUS does not decrease depressive symptoms but TUS may decrease rumination. It was also found that TUS decreased rumination compared to
sham for high worriers but not for low worriers. These exploratory findings suggest that TUS may impact repetitive thought only, or more significantly, for those individuals experiencing high levels of rumination. Depressive symptoms may be more easily impacted for those experiencing lower levels of repetitive thought and overall depression severity. Due to the small sample size of these studies, these conclusions are only possible suggestions of what future research may confirm. In other words, in a sample of 24 participants, groups often reduce to eight to nine participants. As such, the suggestion that level of repetitive thought may influence response to TUS should be investigated in future research with a significantly larger sample size.

It was found that the sham condition slightly increased resting frontal asymmetry while stimulation did not affect frontal asymmetry scores. However, resting EEG after the ultrasound was collected after resting periods and mood assessments (nearly an hour later). As such, it remains unknown whether significant changes in frontal asymmetry occur more directly after TUS stimulation. Future research may examine how frontal asymmetry changes directly after and during TUS stimulation to glean a further understanding of whether and how TUS stimulation impacts frontal asymmetry.

There are a number of limitations of the present work. First, the power delivered by the TUS transducer was substantially lower than in previous studies, perhaps leading to an underpowered TUS intervention. Additionally, this study was designed as an intervention for depressive symptoms. Accordingly, participants were recruited for the present study on the basis of experiencing mild to moderate depressive symptoms; anxiety and worry measures were collected but were not specifically randomized. Contrary to prediction, TUS did not decrease depressive symptoms but instead anxiety
symptoms, and in particular worry, were decreased as a function of TUS stimulation during the course of the intervention. However, as anxiety symptoms were not a basis for randomization, anxiety levels differed between the stimulation and sham groups. Additionally, we included an empirically validated state rumination (past-focused repetitive thought typically associated with depression) but not a state worry (future-focused repetitive thought typically associated with anxiety) or state anxiety measure.

Although we were able to examine the calm and tense items from the VAMS, future research examining an empirically-validated measure of state anxiety or worry would greatly improve our understanding of whether TUS may improve anxiety. Additionally, future research may recruit based on anxiety rather than depressive symptoms in order to examine whether TUS may serve as an intervention for anxiety disorders. The present findings suggest that more research examining TUS as a potential intervention may be warranted. This work has the potential to lead to a portable, low cost, and non-invasive treatment for anxiety disorders.
References


EEG in Healthy Volunteers with a Prototype Ultrasound Device, I(1), 4208470.

electroencephalogram asymmetry in depressed and nondepressed subjects.

*Biological Psychiatry, 18*(7), 753–762. Retrieved from

Ter Haar, G. (2007). Therapeutic applications of ultrasound. *Progress in Biophysics and
Molecular Biology, 93*(1-3), 111–129. doi:10.1016/j.pbiomolbio.2006.07.005

differences in anterior brain asymmetry and fundamental dimensions of emotion.


Properties of Resting Anterior EEG Asymmetry: Temporal Stability and Internal

reliability of frontal EEG asymmetry scores. *Psychophysiology, 46*(1), 132–42.

doi:10.1023/A:1023910315561

different energies on the conduction properties of neural tissue. *Ultrasonics, 43*(7),


Administration, F. and D. (1999). Summary of Safety and Effectiveness, 92(c), PMA:


electroencephalogram asymmetry in depressed and nondepressed subjects.


Tufail, Y., Matyushov, A., Baldwin, N., Tauchmann, M. L., Georges, J., Yoshihiro, A.,


<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>T-test comparing Sham versus Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI</td>
<td>10-25</td>
<td>18.42</td>
<td>4.34</td>
<td>t(24) = -0.276, p = 0.785</td>
</tr>
<tr>
<td>OASIS</td>
<td>0-12</td>
<td>5.62</td>
<td>2.866</td>
<td>t(24) = 0.208, p = 0.837</td>
</tr>
<tr>
<td>PSWQ</td>
<td>33-69</td>
<td>53.29</td>
<td>10.748</td>
<td>t(24) = 2.194, p = 0.039</td>
</tr>
<tr>
<td>RRS</td>
<td>29-63</td>
<td>45.42</td>
<td>9.67</td>
<td>t(24) = 2.532, p = 0.019</td>
</tr>
</tbody>
</table>

*Table 1.* This table shows descriptive statistics for all participants included in analyses.
Table 2. This table shows the correlation between changes from Day 5 to Day 1 in all major outcome variables and Stimulation condition, separated by potential moderators. Negative correlations indicate that those in Sham condition had greater change, whereas positive scores indicate that those in the Stimulation condition had greater change. A nondependent samples difference in correlation was conducted. All Z scores above 1 are highlighted and explicated in the above text.
Participated in Introductory Psychology Survey Pool
N=3,147

Participants Scoring Between 9 and 27 Were Contacted Via E-mail to Complete Online Screening
N=1,191

Began Online Screening
N=410

Excluded after screening (N=355)
- Ran out of time to contact (N=108)
- BDI too low (N=95)
- BDI too high (N=39)
- Severe headaches (N=29)
- No longer interested (N=21)
- Other treatment (N=20)
- Concussion (N=19)
- Left-handed (N=12)
- Out of age range (N=7)
- Serious medical illness (N=5)

Withdraw From Survey
N=29

Began Laboratory Sessions
N=26

Excluded and Replaced (N=2)
- Atypical presentation (N=1)
- Dropped after Day 1 (N=1)

Randomly Assigned to Sham
N=14

Randomly Assigned to Stimulation
N=12

Sham Condition Included in Analysis
N=12

Completed One Month Follow-up
N=6

Stimulation Condition Included in Analysis
N=12

Completed One Month Follow-up
N=7
*Figure 1.* The above Consort chart shows the recruitment procedure and drop-out for the present study. Due to matching by age, sex, and BDI level, a number of participants were excluded after screening because they did not match someone else by age, sex, or BDI level. Additionally, one participant who completed the study through to Day 5 was excluded because her BDI scores varied over 30 points, well above 2 SDs of change, due to significant life events during the course of the study (see text for more explanation).
Figure 2. This figure indicates the location where the ultrasound probe was placed, which has been previously shown to increase positive mood in humans (Sanguinetti, 2014). The image models where energy delivery would be focused in the brain. Both the TUS stimulation condition and TUS sham condition (no power) were administered identically at this location, blind to participants and researchers.
Figure 3. This figure shows the structure of the study in a graphical timeline. For sessions including EEG (days 1, 5, and 10), resting EEG recordings will occur first and last to parallel the structure of sessions without EEG. Trait measures will include the BDI-II and OASIS each day. On days marked with a star trait measures will also include the RRS and PSWQ. State mood and state rumination will be assessed each day. TUS indicates point at which TUS Stimulation Condition or Sham Condition was administered.
Figure 4. There was a medium effect of condition on worry change from Day 5 to 1, such that those in the stimulation condition had decreased worry over the course of the intervention compared to those in the placebo, $t=-1.742, p=0.097$. Those in the stimulation condition, on average, had decreased worry over the course of the intervention ($M=-1.83, SD=7.73$), whereas those in the sham condition had increased levels of worry at day 5 compared to day 1 ($M=3.30, SD=5.67$).
Figure 5. Effect of Condition on BDI change from Day 1 to Day 5, separately for never depressed and previously depressed participants. Prior depression status was determined based on the self-report SCID. Although there was only a negligible differential effect of Condition among Never Depressed participants, a medium effect of Condition was observed for Previously Depressed participants (r = -.523, p = .121), where the Sham Condition decreased symptoms from Day 1 to Day 5 more than the Stimulation Condition.
Figure 6. Change in Global Affect and Global Vigor 10 minutes and 30 minutes after Stimulation across all days. Stimulation Condition compared to Sham Condition produces a greater short-term increase in positive affect 10-minutes following stimulation.
Figure 7. Global Vigor decreased in TUS stimulation more than during sham, F=3.481, p=0.06. Follow-up analysis (see text) revealed that this appeared to be due to a reduction in state anxiety among those receiving Stimulation Condition but not Sham Condition.
Appendix 1

Has there been a period within the last month when you were feeling depressed or down most of the day nearly every day for at least two weeks?
☐ Yes ☐ No

Has there been a period in the last month when you lost interest or pleasure in most things nearly every day for at least two weeks?
☐ Yes ☐ No

IF NO TO BOTH, SKIP TO SECTION B.

If yes to either of the above, focus on the worst two weeks of feeling depressed or down/losing interest. Mark any statements that apply for this two week period:

**Note that no statements may apply.

Appetite
☐ My appetite decreased nearly every day
☐ My appetite increased nearly every day
☐ I ate less than usual nearly every day
☐ I ate more than usual nearly every day
☐ I lost weight
☐ I gained weight

Sleep
☐ I had more trouble falling asleep
☐ I woke up more frequently throughout the night
☐ I woke up earlier than is normal for me
☐ I was sleeping less than is usual for me nearly every night
☐ I was sleeping more than is usual for me nearly every night

Movement
☐ I was so fidgety or restless I was unable to sit still nearly every day
☐ I talked or moved more slowly than is normal for me nearly every day

Energy
☐ I felt tired all day nearly every day
☐ I dragged throughout the day nearly every day

Self-concept
☐ I felt differently about myself nearly every day
☐ I felt worthless nearly every day
☐ I felt guilty about little things I had done or not done nearly every day

Concentration
☐ I had trouble thinking or concentrating nearly every day
☐ Difficulty concentrating interfered with school, work, or relationships
☐ I found it difficult to make decisions about everyday things nearly every day

Suicide
☐ I was thinking a lot about death
☐ I was thinking I would be better of dead
☐ I was thinking about hurting myself
☐ I attempted to hurt myself
☐ I attempted suicide

Did this period of feeling down make it hard for you to do your work, take care of things at home, or get along with other people?
☐ Yes ☐ No
If yes, in what area did it interfere?

Have there been any other times when you were feeling this down?
☐ Yes ☐ No
If yes, how many distinct times throughout your life have you felt this down?

SECTION B.

Have you ever had a period of time in your life when you were feeling depressed or down most of the day nearly every day for at least two weeks?
☐ Yes ☐ No

Have you ever had a period of time when you lost interest or pleasure in most things nearly every day for at least two weeks?
☐ Yes ☐ No

If yes to either of the above, focus on the worst two weeks of feeling depressed or down/losing interest. Mark any statements that apply for this two week period:

**Note that no statements may apply.**

Appetite
☐ My appetite decreased nearly every day
☐ My appetite increased nearly every day
☐ I ate less than usual nearly every day
☐ I ate more than usual nearly every day
☐ I lost weight
☐ I gained weight

Sleep
☐ I had more trouble falling asleep
☐ I woke up more frequently throughout the night
☐ I woke up earlier than is normal for me
☐ I was sleeping less than is usual for me nearly every night
☐ I was sleeping more than is usual for me nearly every night

Movement
☐ I was so fidgety or restless I was unable to sit still nearly every day
☐ I talked or moved more slowly than is normal for me nearly every day

Energy
☐ I felt tired all day nearly every day
☐ I dragged throughout the day nearly every day

Self-concept
☐ I felt differently about myself nearly every day
☐ I felt worthless nearly every day
☐ I felt guilty about little things I had done or not done nearly every day

Concentration
☐ I had trouble thinking or concentrating nearly every day
☐ Difficulty concentrating interfered with school, work, or relationships
☐ I found it difficult to make decisions about everyday things nearly every day

Suicide
☐ I was thinking a lot about death
☐ I was thinking I would be better of dead
☐ I was thinking about hurting myself
☐ I attempted to hurt myself
☐ I attempted suicide

Did this period of feeling down make it hard for you to do your work, take care of things at home, or get along with other people?
☐ Yes ☐ No
If yes, in what area did it interfere?

Have there been any other times when you were feeling this down?
☐ Yes ☐ No
If yes, how many distinct times throughout your life have you felt this down?
Appendix 2

I enjoyed participating in this treatment study.

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
</table>

I would recommend this treatment to other people.

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
</table>

I feel that my mood improved from this treatment study.

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
</table>

I feel that I had fewer uncontrollable, negative thoughts after this treatment study.

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
</table>