Reduction of Anxiety Related Symptoms Using Low Intensity Ultrasound Neuromodulation on the Auricular Branch of the Vagus Nerve; A Preliminary Study

Izzy Kohler, Jon Hacker, Ethan Martin

Submitted to: JMIR Neurotechnology
on: December 07, 2024

Table of Contents

Reduction of Anxiety Related Symptoms Using Low Intensity Ultrasound Neuromodulation on the Auricular Branch of the Vagus Nerve; A Preliminary Study

Izzy Kohler[1] DPT; Jon Hacker[1] BS; Ethan Martin[1] BS

[1]NeurGear Rochester US

Corresponding Author:
Ethan Martin BS
NeurGear
295 Warrington Dr
Rochester
US

Abstract

Background: Neuromodulation of the auricular branch of the vagus nerve using low intensity focused ultrasound (LIFU) is an emerging mode of treatment for anxiety that could provide a complementary or alternative treatment modality for individuals that are refractory to conventional interventions. The proposed benefits of this technology have been largely unexamined with clinical populations. Further research is required to understand its clinical potential and utility in improving and managing moderate to severe symptoms.

Objective: The aim of this study was to do a preliminary investigation into the efficacy, safety, and usability of the wearable headset that delivers LIFU to the auricular branch of the vagus nerve for the purpose of alleviating anxiety disorder symptoms.

Methods: This study was a pre-post intervention study design for which we recruited 28 participants with a Beck Anxiety Inventory score greater than or equal to 16 points. Participants completed five minutes of treatment daily consisting of low intensity focused ultrasound neuromodulation delivered to the auricular branch of the vagus nerve. Participants did this for a period of four weeks. Assessments of anxiety symptom severity (Beck Anxiety Inventory), depression symptom severity (Beck Depression Inventory), post-traumatic stress disorder symptom severity (Post Traumatic Stress Disorder Checklist for the DSM-5), and sleep quality (Pittsburgh Sleep Quality Index) were taken prior to starting treatment and weekly for the four weeks of treatment. Usability and safety were also assessed using an exit questionnaire and adverse event logging.

Results: After completing four weeks of low intensity focused ultrasound neuromodulation to the auricular branch of the vagus nerve the average Beck Anxiety Inventory score decreased 14.9±10.6 points (Cohen d=1.06, p <.001), the average Beck Depression Inventory score decreased 10.3±7.8 points (Cohen d=0.81, p<.001), the average Post Traumatic Stress Disorder Checklist for the DSM-5 score decreased 20.0±20.5 points (Cohen d=0.94, p<.001), and the average Pittsburgh Sleep Quality Index score decreased 2.2±3.1 points (Cohen d=0.65, p=.001). On the exit questionnaire participants rated the treatment highly for ease of use, effectiveness, and worthiness of the time invested. Only one adverse event was reported throughout the entire trial, which was mild and temporary.

Conclusions: This preliminary study provided justification for further research into the efficacy, safety, and feasibility of using LIFU to modulate the auricular branch of the vagus nerve and reduce the symptoms of anxiety, depression, and PTSD. Clinical Trial: ClinicalTrials.gov [NCT06574971]

(JMIR Preprints 07/12/2024:69770)
DOI: https://doi.org/10.2196/preprints.69770

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✓ **Please make my preprint PDF available to anyone at any time (recommended).**
Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users. Only make the preprint title and abstract visible.

No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✔ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**

Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v

Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http

Original Manuscript

Reduction of Anxiety Related Symptoms Using Low Intensity Ultrasound Neuromodulation on the Auricular Branch of the Vagus Nerve

Izzy Kohler, DPT[1]*, Jon Hacker, BS[1], Ethan Martin, BS[1]
[1]NeurGear, Rochester, New York, USA
*Send correspondence to
Izzy Kohler, DPT
NeurGear, Inc, Rochester, New York, USA, Email: kohler@neurgear.com

Abstract

Background
Neuromodulation of the auricular branch of the vagus nerve using low intensity focused ultrasound (LIFU) is an emerging mode of treatment for anxiety that could provide a complementary or alternative treatment modality for individuals that are refractory to conventional interventions. The proposed benefits of this technology have been largely unexamined with clinical populations. Further research is required to understand its clinical potential and utility in improving and managing moderate to severe symptoms.

Objective
The aim of this study was to do a preliminary investigation into the efficacy, safety, and usability of the wearable headset that delivers LIFU to the auricular branch of the vagus nerve for the purpose of alleviating anxiety disorder symptoms.

Methods
This study was a pre-post intervention study design for which we recruited 28 participants with a Beck Anxiety Inventory score greater than or equal to 16 points. Participants completed five minutes of treatment daily consisting of low intensity focused ultrasound neuromodulation delivered to the auricular branch of the vagus nerve. Participants did this for a period of four weeks. Assessments of anxiety symptom severity (Beck Anxiety Inventory), depression symptom severity (Beck Depression Inventory), post-traumatic stress disorder symptom severity (Post Traumatic Stress Disorder Checklist for the DSM-5), and sleep quality (Pittsburgh Sleep Quality Index) were taken prior to starting treatment and weekly for the four weeks of treatment. Usability and safety were also assessed using an exit questionnaire and adverse event logging.

Results
After completing four weeks of low intensity focused ultrasound neuromodulation to the auricular branch of the vagus nerve the average Beck Anxiety Inventory score decreased 14.9±10.6 points (Cohen *d*=1.06, *p* <.001), the average Beck Depression Inventory score decreased 10.3±7.8 points (Cohen *d*=0.81, *p*<.001), the average Post Traumatic Stress Disorder Checklist for the DSM-5 score decreased 20.0±20.5 points (Cohen *d*=0.94, *p*<.001), and the average Pittsburgh Sleep Quality Index score decreased 2.2±3.1 points (Cohen *d*=0.65, *p*=.001). On the exit questionnaire participants rated the treatment highly for ease of use, effectiveness, and worthiness of the time invested. Only one adverse event was reported throughout the entire trial, which was mild and temporary.

Conclusions
This preliminary study provided justification for further research into the efficacy, safety, and feasibility of using LIFU to modulate the auricular branch of the vagus nerve and reduce the symptoms of anxiety, depression, and PTSD.

Introduction

Anxiety is the “anticipation of real or imagined future threat or danger” (Penninx et al., 2021) which manifests itself with a mix of emotional signals, such as hyperarousal and panic, and physiological ones,

including increased heart rate, shortness of breath, sweating, and chest pain (Chand & Marwaha, 2023 Apr 24). The emotional and physiological responses experienced with anxiety result from activation of the hypothalamus which engages the sympathetic nervous system (SNS) (Tasker & Joëls, 2015). This sympathetic activation is adaptive in short bursts and enables us to handle threats and stressors, but in anxiety disorders, the SNS may be overly sensitive or chronically activated, leading to distress and health challenges over time (Holwerda et al., 2018). Clinically significant anxiety symptoms are disproportionate to the future threat, endure after it has passed, and cause substantial distress or incapacitation (Penninx et al., 2021, Ströhle et al., 2018). The etiology of anxiety disorders is complex, with heritability ranging from 30-67% depending on the research study and anxiety disorder type (Penninx et al., 2021). However, trauma, chronic stress, and other environmental factors play an important role in the development of maladaptive anxiety (Ströhle et al., 2018).

The complex etiology of anxiety opens opportunities for intervention at multiple points in the course of the illness from a variety of disciplines. There are also several multi–disciplinary approaches that offer a more holistic care plan. The primary goal of preventative strategies is to lower the risk of developing disordered anxiety responses prior to onset. Preventative psychoeducational interventions for adolescents and adults have been shown to reduce the risk of anxiety onset (Stockings et al., 2016) with small to moderate effect sizes (Penninx et al., 2021, Stockings et al., 2016), however studies of these interventions tend to end their follow-ups after only 9 months so the long term stability of their benefits after intervention completion is still in question (Penninx et al., 2021). Once an active anxiety disorder has developed, psychotherapeutic treatments for it range in intensity from self-guided programs to highly intense weekly sessions with a licensed therapist. Self-guided treatments derived from evidence-based psychotherapies are more effective than active controls but show smaller effect sizes compared to therapist-guided programs (Fischer et al., 2020). Cognitive behavioral therapy (CBT) is widely considered to be the gold standard for anxiety disorder treatment, particularly in adults, although Haller et al. (2021) found mindfulness-based cognitive therapy and acceptance and commitment therapy to be similar in efficacy. In recent years, virtual psychotherapy modalities have emerged as a compromise that balances the convenience of self-help approaches and the rigor and guidance of a traditional in-person therapy session. Thus, recent advances in telehealth have paved the way for approaches that afford convenience and accessibility without a loss of efficacy (Chi et al., 2022; Moghimi et al., 2023).

Pharmacotherapy is similar in efficacy to psychotherapy, and both pharmacotherapy and psychotherapy are considered first-line treatments for anxiety disorders in most standard care plans (Penninx et al., 2021). Selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), benzodiazepines, antipsychotics, and beta blockers are all used to treat anxiety. Despite this wealth of options, anxiety disorders remain chronic and refractory to treatment in many individuals, with 15-40% achieving less than 50% remission in symptoms (Garakani et al., 2020). Studies of combinations of psychotherapeutic and pharmacological approaches to anxiety treatment are sparse, leaving confusion surrounding which combinations are most efficacious (Penninx et al., 2021). Taken as a whole, while current neurobiological and psychosocial treatment approaches to anxiety disorders are sufficient for a large portion of affected individuals, there is still a substantial proportion of patients who would benefit from additional treatment options.

Low intensity focused ultrasound (LIFU) is an emerging mode of treatment for anxiety that could provide an alternative treatment modality. LIFU can stimulate or inhibit neural activity, depending on the parameters of the energy applied to neural tissue. Also referred to as acoustic neuromodulation, the use of LIFU to modulate the activity of neural structures is a promising method for non-invasive treatment of neurological disorders (Baek et al., 2017). While the majority of investigations featuring LIFU neuromodulation have primarily focused on modulation of neural structures within the central nervous system (CNS), disorders affecting the peripheral nervous system (PNS) stand to benefit from this powerful tool as well (Collins & Mesce, 2022). LIFU neuromodulation of the PNS is accomplished through a non-thermal, non-cavitation bio-

effect produced by setting the parameters to the intermediate intensity range. At intensities between 1-200 W/cm^2, ultrasound is able to non-invasively and reversibly enhance peripheral neural activities by activating low-threshold mechanosensitive nerve endings, opening mechanosensitive ion channels to evoke action potentials (Collins & Mesce, 2022). Ultrasound of intermediate intensity also enhances the neural activity of peripheral nerve axons, leading to increased nerve conduction velocities in both A- and C-type fibers, which is likely caused by mechanical gating of other ion channels (Feng et al., 2019). In addition, enhanced neural activity could be attributed to a direct effect of acoustic radiation forces on the lipid-bilayer neural membrane. Plausible mechanisms for this include a transient capacitive current from rapid changes of local membrane capacitance and transmembrane pore formation to allow sodium and potassium ions to pass through (Collins & Mesce, 2022; Feng et al., 2019).

The vagus nerve, also known as cranial nerve X, is the longest cranial nerve and its branches enable the organs to adjust to the demands of a person's internal state and external environment. The vagus nerve is a primary component of the parasympathetic nervous system, which, paired with the sympathetic nervous system, constitutes the autonomic nervous system (Johnson & Wilson, 2018). Normally, sympathetic and parasympathetic nerve pathways act synergistically to create a state of equilibrium appropriate to meet the demands of the current internal state and external challenges. Disruption of the balance of sympathetic and parasympathetic activity in favor of sympathetic activity is one indicator of anxiety disorders (Martin et al., 2009).

The many branches of the vagus nerve are increasingly seen as pathways for promoting or restoring health and ameliorating the physiologic unease that gives rise to anxiety and other negative mental states (Kaniusas et al., 2019). The vagus nerve operates bidirectionally, meaning states of homeostasis and calm can be induced from the bottom up or the top down. The brain can employ cognitive strategies to dissipate states of bodily unease (top down) or activate vagal nerve pathways to create psychological comfort and a sense of safety (bottom up) (Butt et al., 2020). In addition to its role in regulating the parasympathetic nervous system, the vagus nerve also projects to the amygdala and hippocampus, both of which are important to extinction learning techniques commonly used in the treatment of anxiety and PTSD (Breit et al., 2018; Noble et al., 2017). Stimulation of the vagus nerve can down-regulate sympathetic activity, , restoring visceral order and psychological calm (George et al., 2008).

Early research into the clinical applications of vagus nerve stimulation (VNS) primarily centered on epilepsy and depression (Johnson & Wilson, 2018), but the vagus nerve is an attractive target for anti-anxiety therapies as well. In addition to its role in regulating the parasympathetic nervous system, the vagus nerve also projects to the amygdala and hippocampus, both of which are important to extinction learning techniques commonly used in the treatment of anxiety and PTSD (Breit et al., 2018; Noble et al., 2017). Preliminary clinical studies have demonstrated VNS's therapeutic applications to treatment-resistant anxiety disorders (George et al., 2008) and long COVID-19 symptoms (Badran et al., 2022). Physiological changes as an effect of vagus nerve stimulation are also well known in the literature. Wittbrodt et al. (2021) discovered that transcutaneous cervical vagus nerve stimulation (tcVNS) increased activation of the anterior cingulate and hippocampus during exposure to traumatic scripts. Lamb et al. (2017) found that transcutaneous auricular vagal nerve stimulation (taVNS) improved respiratory sinus arrhythmia and skin conductance during exposure to physical and emotional stress. Bremner et al. (2019) found tcVNS decreased inflammatory markers and sympathetic tone while increasing medial prefrontal function during exposure to trauma-specific and neutral stressors.

While VNS is traditionally done electrically, ultrasound's noninvasiveness and specificity make it ideal for VNS (Riis & Kubanek, 2022). Ultrasound has been successfully used for vagus nerve neuromodulation in rats (Juan et al., 2014) and for peripheral nerve (Riis & Kubanek, 2022) and sub-organ (Cotero et al., 2019) stimulation in humans. With a recent study showing the feasibility of transauricular VNS as an at-home intervention (Badran et al., 2022), transauricular ultrasound VNS has emerged as a non-invasive, yet potentially effective,

at-home treatment for the management of anxiety symptoms. In response to this, we have developed a wearable headset with an ultrasound transducer that delivers LIFU to the auricular branch of the vagus nerve that can be used at home for treatment of anxiety symptoms. The purpose of this study was to do a preliminary investigation into the efficacy, safety, and usability of the wearable headset that delivers LIFU to the auricular branch of the vagus nerve for the purpose of alleviating the symptoms of anxiety. Because depression (Bentley et al., 2021) and PTSD (Williamson et al., 2021) frequently co-occur with anxiety, we also investigated the efficacy of transauricular ultrasound VNS for alleviating the symptoms of depression and PTSD in individuals with anxiety.

Methods

Study Design

This was a pre-post-intervention study in which all participants received the intervention daily, at home, for a period of four weeks. The clinical trial is registered at ClinicalTrials.gov [NCT06574971]. Informed consent was obtained from each of the 28 participants prior to screening. All activities were completed remotely and a ZenBud device with a user manual and participant instructions was shipped to each participant's home. Participants completed five minutes of low intensity focused ultrasound to the auricular branch of the vagus nerve each day using the ZenBud device. Treatment could be completed at any convenient time of day and did not have to be completed at the same time every day, as long as the treatment was completed within every 24-hour period. Assessments were completed online on the day before the first treatment session, and then weekly. The final assessment was completed on the day of the final treatment after the final treatment session. The battery of assessments included four validated clinical outcome measures, the Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), PTSD Checklist for DSM-5 (PC5), and the Pittsburgh Sleep Quality Index (PSQI). The details of these assessments are further described in the data collection section.

Participant Recruitment

Adults in the United States were recruited through online social media advertising mentioning a study investigating a new treatment for anxiety disorders. Interested individuals filled out a study registration form containing only contact information and were then contacted by a member of the research team via email with further details of the study and a link to sign the informed consent. Upon completion of the informed consent, candidates were then screened for inclusion and exclusion criteria using online questionnaires. Interested individuals were included if they scored 16 or higher on the Beck Anxiety Inventory, were over the age of 18, and did not have any additional conditions that were contraindications for vagus nerve stimulation or ultrasound. Conditions that were contraindications for vagus nerve stimulation included a history of a vagotomy, heart arrhythmias, schizophrenia, or rapid cycling bipolar disorder. Conditions that were contraindications for ultrasound included presence of a pacemaker, pregnancy, active cancer, decreased sensation or open wounds in the ear, ear infection, or metal implants in or around the ear. A BAI score of 16 was chosen as the cutoff threshold because a score of 16 or higher in the BAI classifies an individual as having moderate to severe anxiety symptoms (Fydrich et al., 1992). We did not exclude individuals who were receiving other treatments for their anxiety as long as the treatment was not initiated or ceased within the past month.

A total of 100 individuals completed the interest form, 63 signed the informed consent and were screened, and 28 were enrolled in the study. Each participant was assigned a unique identifier code so that participant information could be managed in a confidential manner throughout the study and the data could be de-identified upon completion of the study. Only the principle investigator and the study coordinator had access to the unique identifier code assignments

Ultrasound Device

The Zenbud, the device used for this trial, is a proprietary CE-compliant over-the-ear wearable headset that was developed by NeurGear (Rochester, New York; **Figure 1**). The ZenBud delivers low-intensity focused ultrasound to the auricular branch of the vagus nerve through several layers of skin. The ZenBud is designed to mimic a standard headset so that users can integrate the use of the device into their routine with minimal effort and discomfort. When the user plugs the ZenBud device into the battery pack it immediately turns on. There is a hardware limit in the circuitry so that the device shuts down after running for 29 minutes, limiting the duration of use. The ZenBud device specifications include a center frequency of 5.3 megahertz, a pulse repetition frequency of 41 hertz, a duty cycle of 50%, and an average intensity of 1.03 megapascals.

(a) (b)

Figure 1*: (a) The ZenBud headset, powerpack, power brick, and bottle of gel. The ultrasound transducer is located in the round earpiece on the right side of the headset (b) The ZenBud device as depicted properly placed on a model human head.*

A detailed instruction manual was provided in the package with every device. A copy of the manual is provided as supplementary materials. The participants were instructed to use the device once a day for five minutes unless instructed otherwise by a healthcare professional. There were no stipulations set for the time of day that treatment could be completed, participants were free to choose a time that was convenient for them. For step-by-step set up and use, participants were instructed to apply a pea sized amount of the aquasonic gel to the blue part of the device located directly above the headset (**Figure 2a**), position the blue circular pad against the skin just above the ear canal (**Figure 2c**), adjust the headset until they felt a moderate pressure (without pain) just above the ear canal where the blue circular pad was positioned (**Figure 2b**), and begin stimulation by plugging the USB cable into the battery pack (**Figure 2d**). Once the headset is plugged into the battery pack the device starts working and a low humming noise can be heard. The manual instructs users to listen for the humming sound to indicate the device is working properly.

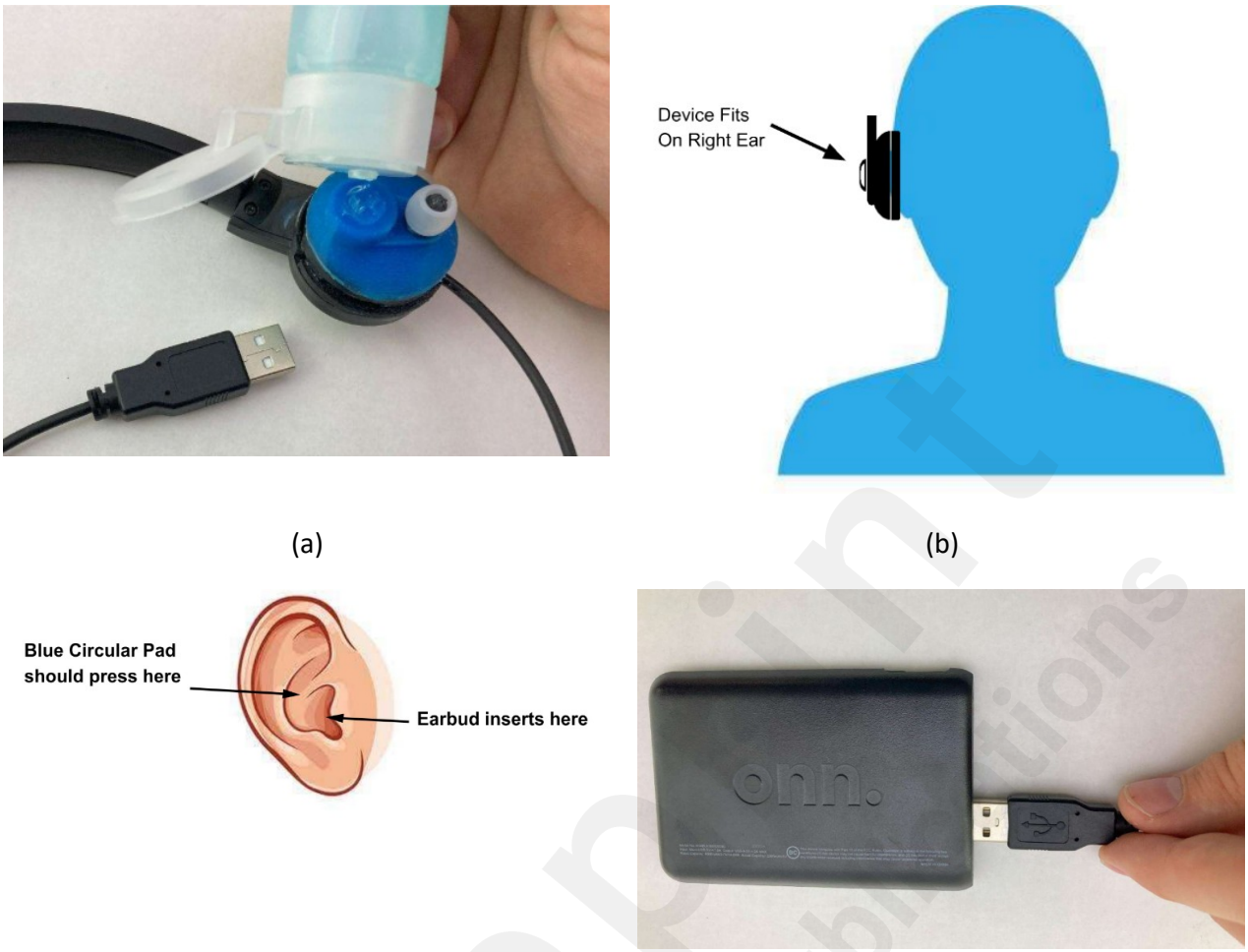


Figure 2. *Images extracted from the ZenBud user manual depicting step-by-step set up and operation of the device. (a) Application of the ultrasonic gel (b) Placement of the headset with the headset located over the right ear (c) Correct placement of the headset on the ear (d) Treatment is started upon inserting the USB cable into the battery pack.*

Data Collection

Assessments were done using a battery of four validated clinical outcome measures. These were taken on the day before the first treatment session, weekly, and on the day of the final treatment session after the final treatment session was completed. The following four clinical outcome measures were used.

Beck Anxiety Inventory (BAI)

The BAI is a rating scale used to evaluate the severity of anxiety symptoms in individuals ages 17 and up. It contains 21 self-report items that reflect common physiological symptoms of anxiety such as numbness or tingling, feeling hot, and trembling. Participants indicate how much they have been bothered by each symptom, from “not at all” to “severely”, using a four-point Likert scale. The item scores are then summed, with possible scores ranging from 0-63. A total score of 0–7 is classified as minimal anxiety, 8–15 as mild, 16–25 as moderate, and 26–63 as severe (Beck et al, 1988; Beck & Steer, 1990). The BAI has a Cronbach’s alpha of 0.91 , a good test-retest reliability (kappa = 0.65 (95% CI[0.61,0.69]), and correlates moderately (Pearson r

= 0.51) with the revised Hamilton Anxiety Rating Scale (Bardhoshi et al., 2016; Beck et al., 1988; Fydrich et al., 1992).

Beck Depression Inventory Version II (BDI-II)

Depression and anxiety are highly comorbid, with 60% of patients with anxiety disorders also having depression (Bentley et al., 2021). Long-term activation of the stress response may explain this overlap (Maletic et al., 2007), implying that inhibiting over-activation of the stress response may alleviate depressive symptoms in addition to anxiety and stress. The BDI-II is a valid and reliable self-report measure for depression that quantifies depressive symptoms over the last week (Beck et al., 1961). For each of the 21 items, respondents are asked to choose the statement they most agree with out of a group of four choices. Each statement corresponds to a score ranging from 0-3, and total scores range from 0-63 (Beck et al., 1988; Beck et al., 1996; Wang & Gorenstein, 2013). The scores are classified as minimal depression (0-13), mild depression (14-19), moderate depression (20-28), and severe depression (29-63) (Beck et al., 1996). The BDI is positively correlated with the Hamilton Depression Rating Scale with a Pearson *r* of 0.71, showing good agreement. The test was also shown to have a high one-week test–retest reliability (Pearson's r = 0.93), suggesting that it was not overly sensitive to daily variations in mood and high internal consistency (α = .91) (Beck et al., 1996).

PTSD Checklist for DSM-5 (PCL-5)

While the DSM-5 does not classify PTSD as an anxiety-related disorder, both PTSD and anxiety disorders involve dysregulation in neural structures dealing with fear, arousal, and anticipation of future threats (Williamson et al., 2021). Thus there is reason to believe that VNS simulation could be beneficial for PTSD-related symptoms. The PCL-5 is a self-report questionnaire that helps assess the presence and severity of PTSD symptoms. The PCL-5 can be used to screen for PTSD, assist in making a provisional diagnosis, and monitor symptoms over time. The measure asks participants to rate how much they were bothered by certain PTSD symptoms over the past month on a 5-point Likert scale ranging from “not at all” to “extremely” (Blevins et al., 2015). Total scores range from 0-60 and scores ranging from 31-33 are widely accepted as the cutoff for diagnosing PTSD (Forkus et al., 2023). In a systematic review of PCL-5 validation studies, Forkus et al. (2023) concluded that the full 20-item version showed good to excellent internal consistency across studies (Cronbach’s alphas ranging from 0.83-0.97) and acceptable temporal stability (correlations ≥0.60) across time points within 1-5 weeks of one another. Scores were also moderately to highly correlated with other measures of PTSD as well as measures of anxiety, depression, suicidal ideation, and sleep.

Pittsburgh Sleep Quality Index (PSQI)

Anxiety and sleep disturbance are frequently co-occurring (Cox & Olatunji, 2016) such that sleep disturbance is a DSM-5 criterion for generalized anxiety disorder. Studies have found correlations between BAI scores and subjective sleep quality among college students (KP et al., 2021), indicating that measuring sleep quality could provide insight into the burden of anxiety on well-being. The PSQI is a validated and widely used global measure of sleep quality (Buysse et al., 1989; Mollayeva et al., 2016). It comprises 19 self-report items and 5 items to be reported by a sleeping partner, but the 19 self-report items are commonly used on their own in research contexts (Fabbri et al., 2021). The different items call for responses in different formats (bedtimes, number of hours, Likert scales etc.), thus the instrument is scored with the use of seven component scores which are summed for one total score ranging from 0-21 (Buysse et al., 1989). The original creators of the PSQI found that a score greater than or equal to 5 differentiated between “good” and “poor” sleepers with a sensitivity of 89.6% and a specificity of 86.5% (Buysse et al., 1989). Research since has generally supported the validity of this cutoff. Mollayeva et al., (2016) did a meta-analysis of the psychometric properties of the PSQI and found that it showed acceptable internal reliability for within-group comparisons across studies (Cronbach’s alphas ranging from 0.70 to 0.83). They also found that intraclass correlations for PSQI scores

across timepoints met the cutoffs for use in within-group comparisons (greater than or equal to 0.70) (Mollayeva et al., 2016).

Exit Survey

In addition to the clinical outcome measures, participants also completed an exit survey on the final day of the trial. This survey asked questions regarding overall satisfaction with the treatment, impact on daily functioning and quality of life, ease of use, symptom improvement, side effects, and how quickly effects from the treatment were perceived to be felt. The purpose of this questionnaire was to provide further insight into the perceived experiences of the participants during the treatment period which is important information for full and complete understanding of the treatment's impact.

Adverse Event Tracking

Adverse events (AEs) and device deficiencies were documented and categorized in accordance with ISO14155:2020. These AEs were documented based on reports provided by the participants through email or on the exit survey. The investigators closely tracked the AEs and their resolution throughout the study. Each AE was categorized by type and seriousness according to the definitions provided in ISO14155. Whether an AE was related to the device or procedures was also distinguished. All available details for each AE were recorded in the participant CRFs (case report forms), including relationship to the investigational device, severity (mild, moderate, or severe), onset date, resolution status, any action taken, and if there were any sequelae. For the causality assessment of all AEs, the MDCG 2020-10/1 guideline was followed. This guidance is specifically aimed at severe adverse events (SAE); however, it was extrapolated to all AEs for this study.

According to MDCG 2020-10/1, causal relatedness was defined as an AE associated with the investigational device beyond reasonable doubt. Probably device-related was defined as having a relationship with the use of the investigational device that seems relevant and/or the event cannot be reasonably explained by another cause. Possibly device related was defined as having a relationship with the use of the investigational device that was weak but cannot be ruled out completely. Not device related was defined as an event not having a temporal relationship with the device or not following a known response pattern to the device. The AEs were then further classified into mild, moderate, or severe categories. Mild severity AEs correspond to awareness of easily tolerated and mildly irritating signs or symptoms, with no or minimal loss of time from normal activities; these symptoms are transient and do not require therapy or a medical evaluation. Moderate cases are events that introduce a low level of inconvenience or concern to the participant and may interfere with daily activities; moderate experiences may cause some interference with functioning. Severe cases are events that substantially interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; these events are usually incapacitating.

Statistical Analysis

The primary and secondary endpoints of the study are thoroughly described above. These endpoints included pre to post treatment changes from baseline to the end of treatment at four weeks for the BAI as the primary endpoint, and the BDI, PCL-5, and PSQI as secondary endpoints. Baseline scores were defined as the BAI, BDI, PCL-5, and PSQI scores on the first day of treatment, prior to the first treatment session. The within-group analyses were based on a per-protocol estimand and tested with paired two-tailed t tests, where the normality assumption was confirmed with the Shapiro-Wilk test and alpha was set to 0.05. The effect sizes reported in this paper are based on Cohen's *d* and calculated as the mean score at the end of treatment minus the mean score at baseline, divided by the pooled SD for the two scores. The use of per-protocol estimand ensured that the changes in outcome measures within each treatment arm were reflective

of scenarios where the participants used the treatment as directed and thus included only the participants who were compliant to treatment. The usage criteria for inclusion in the per-protocol analysis was set at 5 to 29 minutes of treatment per day 6 to 7 days per week across the intended four-week treatment period. There were only two missing scores, one in week two and one in week three. Because these data are a time series that exhibits a trendline and the number of missing values was very small, these were filled using a linear interpolation between the score from the previous week and the score from the following week. There was no missing baseline or final scores.

To determine the appropriate sample size a power analysis was performed assuming a dependent t-test with a significance level of 5%, power of 80%, and moderate effect size of 0.6 between pairs. This gave us a necessary sample size of 25 participants. Accounting for a potential drop out rate of 20% gave us a target sample size of 30 participants. All analyses were performed using GraphPad Prism 10.3.0 (507).

Results

Study Participants

Between October 22, 2023 and October 2, 2024, 100 individuals completed the online interest form (**Figure 3**). A total of 63 participants consented to the trial, with 26 of these not satisfying the criteria of having a Beck Anxiety Inventory score equal to or greater than 16 , four not responding to requests to complete the screening questionnaire, and one not responding to requests for confirmation of their shipping address. A total of 32 participants were shipped a device, with three of these not responding to requests to complete the baseline assessments and one participant failing to respond to requests to take the reassessments after week two. In total, 28 participants completed all LIFU sessions and weekly assessments (87.5%). Data for all 28 participants who completed the trial is included in the analysis.

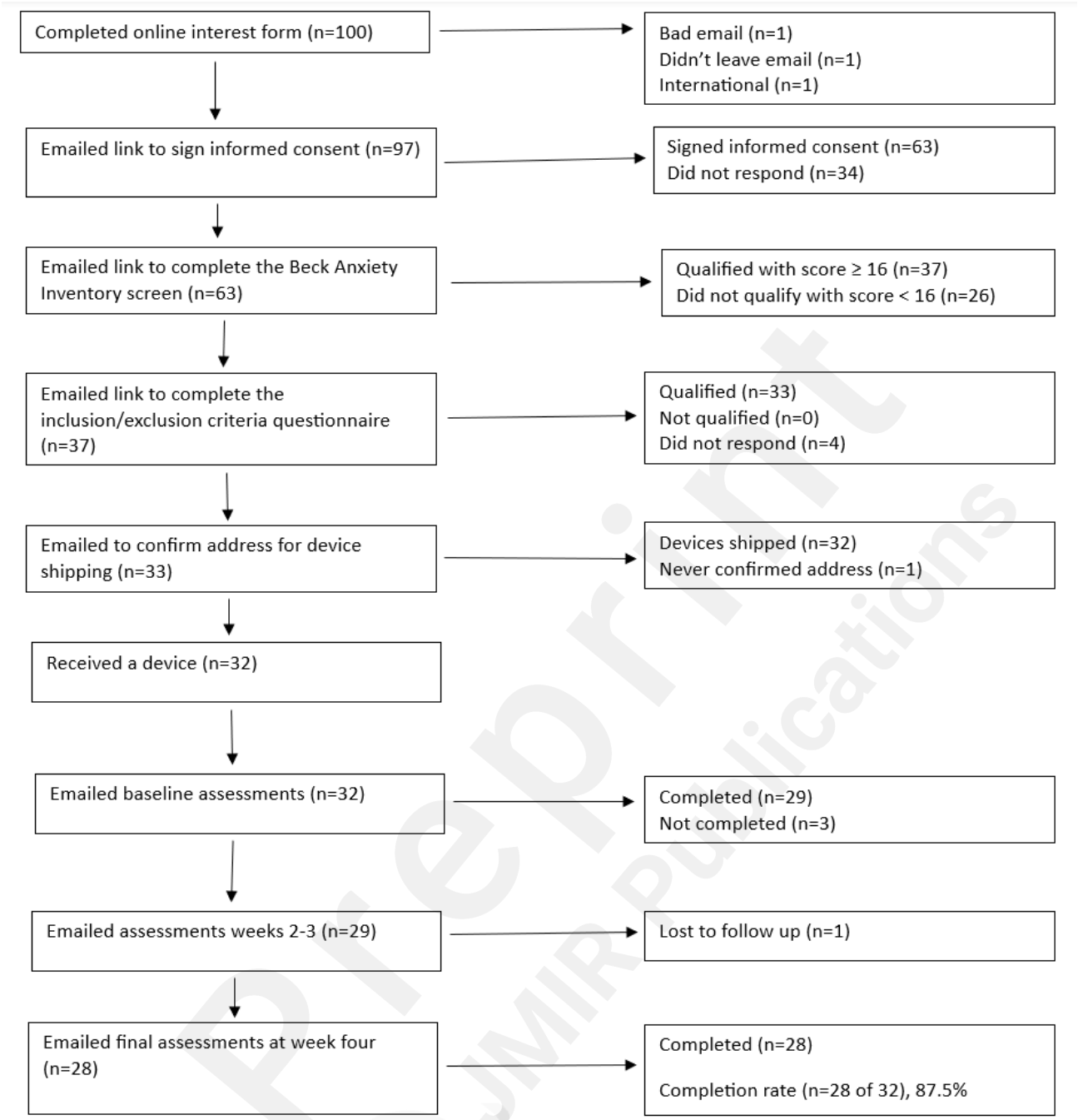


***Figure 3**: Flowchart of study participants through the trial*

The average age of the participants was 48.1±15.6 years. The group was heavily weighted toward women, with 22 women and 6 men. The National Institutes of Health reports that generalized anxiety affects approximately 2.7 percent of American adults, with women experiencing the disorder at a higher rate (3.4 percent) versus men (1.9 percent), making the fact that the sample contained a higher percent of women a reflection of actual population distributions. The self-reported average duration of time suffering with anxiety was 16.5±11.8 years. There were also 8 participants currently receiving treatment for their anxiety and 20 who were not receiving any treatment.

Beck Anxiety Inventory

After four weeks of treatment with the ZenBud, the average BAI score decreased 14.9±10.6 points from 26.5±12.5 to 11.5±11.1 (**Figure 4**). This change in score was both statistically significant (p<.001, two-tailed dependent t-test) and clinically meaningful. While there is no consistently defined definition of clinical improvement for the BAI, based on the categorical definitions of severity for the scores, there was a great deal of progression into decreased severity levels of anxiety throughout the treatment period. As seen on **Figure 5**, at the start of the study, twenty-two participants had BAI scores in the moderate or severe anxiety ranges and only six participants had BAI scores in the mild or minimal severity ranges. After four weeks of using the ZenBud, twenty-two participants had BAI scores into the mild or minimal severity rages, and only six participants had scores in the moderate or severe ranges. In terms of Cohen's *d*, the effect size was large at 1.06.

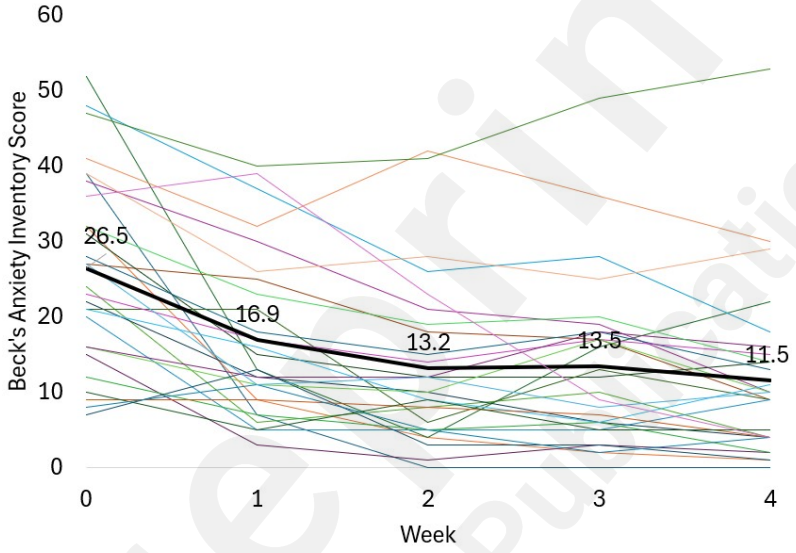


Figure 4. *The progression of Beck Anxiety Inventory scores through four weeks of treatment with ZenBud. The thin lines represent each individual participant. The thick line represents the group mean.*

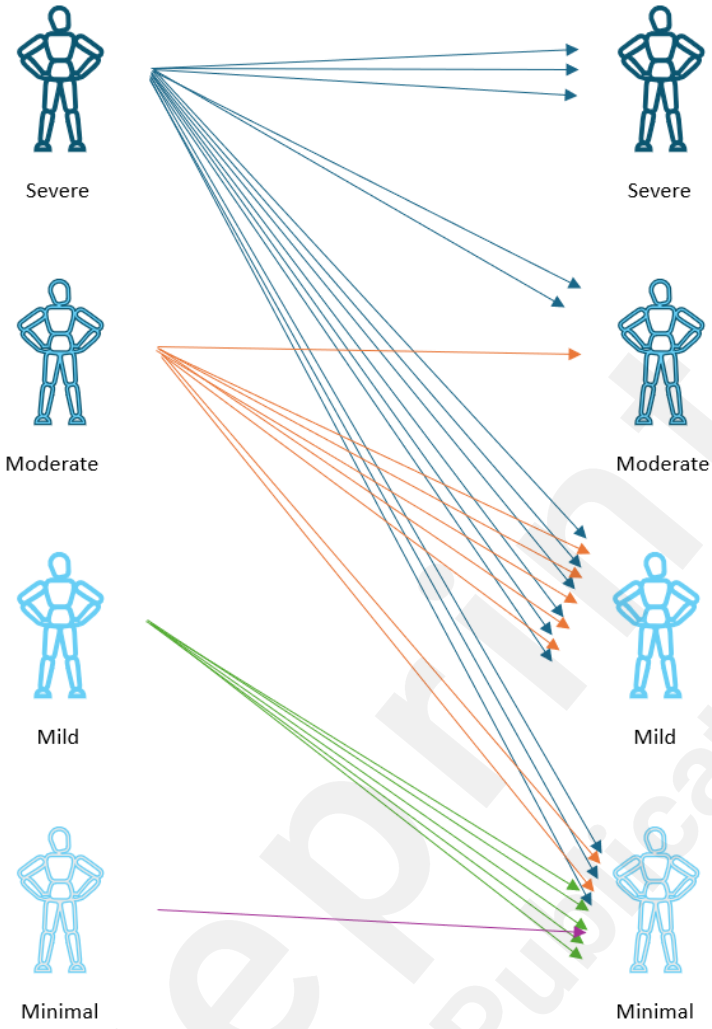


Figure 5. *Categorical movement across degrees of severity based on the Beck's Anxiety Inventory definitions. At the start of the study, twenty participants had BAI scores in the moderate or severe anxiety ranges and only six participants had BAI scores in the mild or minimal severity ranges. After four weeks of using the ZenBud, 20 participants had BAI scores into the mild or minimal severity ranges, and only six participants had scores in the moderate or severe ranges.*

Beck Depression Inventory

After four weeks of treatment with the ZenBud, the average BDI score decreased 10.3±7.8 points from 24.2±10.5 to 13.9±12.6 (**Figure 6**). Similar to results seen for the BAI, this change in score was both statistically significant (p<.001, two-tailed dependent t-test) and clinically meaningful. A 17% reduction in score on the BDI is considered clinically meaningful (Button et al., 2015). Based on this definition, as seen in **Figure 7**, 69.2% of participants demonstrated a clinically meaningful reduction in score by the end of the trial. In terms of Cohen's *d*, the effect size was large at 0.81.

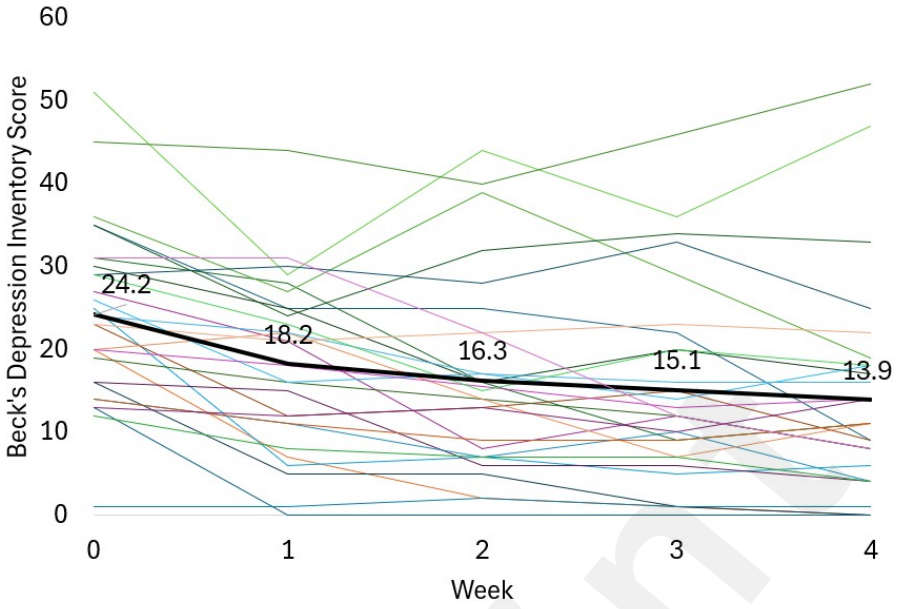


Figure 6. *The progression of Beck Depression Inventory scores through four weeks of treatment with ZenBud. The thin lines represent each individual participant. The thick line represents the group mean.*

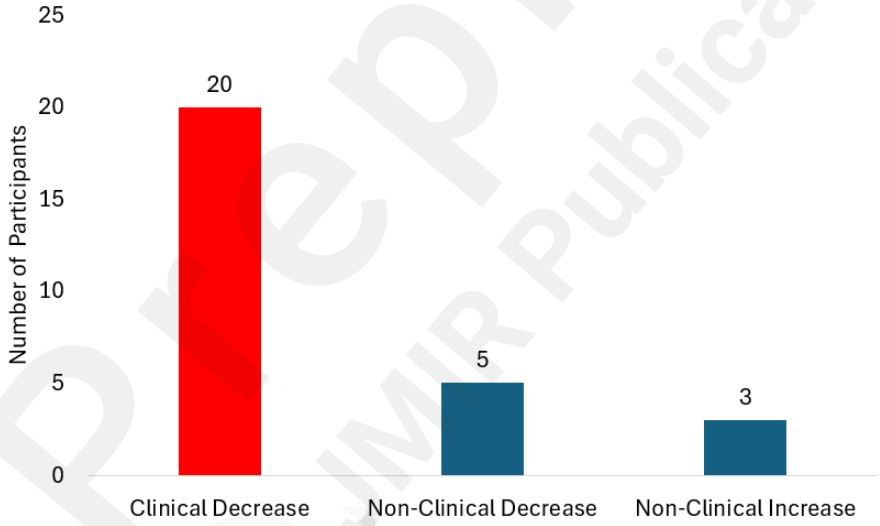


Figure 7. *The number of participants who experienced clinically significant reductions in BDI score following four weeks of treatment with the ZenBud.*

Post Traumatic Stress Disorder Checklist for the DSM-5

After four weeks of treatment with the ZenBud, the average PCL-5 score decreased 20.0±20.5 points from 38.8.8±18.0 to 18.8±18.9 (**Figure 8**). Similar to results seen for the BAI and BDI, this change in score was both statistically significant (p<.001, two-tailed dependent t-test) and clinically meaningful. A 10-point reduction in score on the PCL-5 is considered clinically meaningful (Weathers et al., 2013). Based on this definition, as seen in **Figure 9**, 71.4% of participants demonstrated a clinically meaningful reduction in score by the end of the trial. In terms of Cohen's *d*, the effect size was large at 0.94.

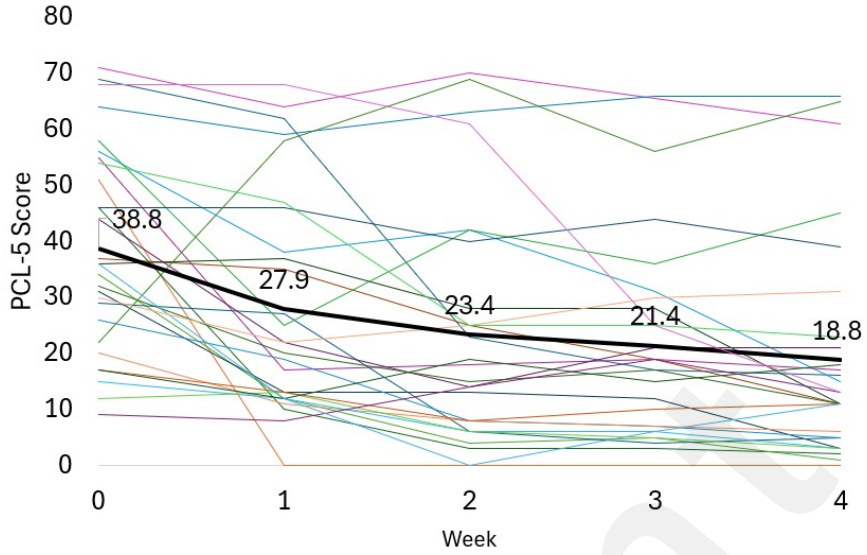


Figure 8. *The progression of PCL-5 scores through four weeks of treatment with ZenBud. The thin lines represent each individual participant. The thick line represents the group mean.*

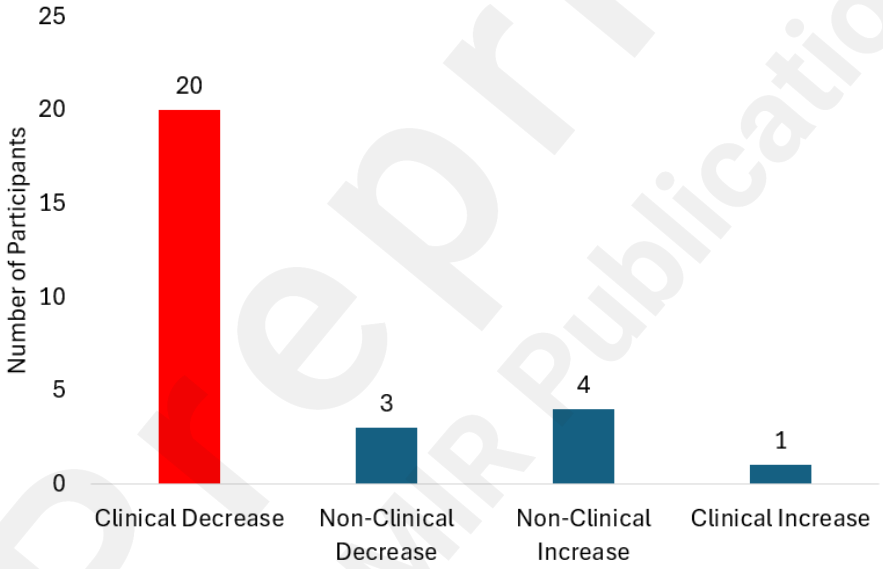


Figure 9. *The number of participants who experienced clinically significant reductions in PCL-5 score following four weeks of treatment with the ZenBud.*

Pittsburgh Sleep Quality Index

After four weeks of treatment with the ZenBud, the average PSQI score decreased 2.2±3.1 points from 12.1±3.2 to 9.9±3.2 (**Figure 10**). While this change in score was statistically significant (p=.001, two-tailed dependent t-test) it was not clinically meaningful. In terms of Cohen's *d*, the effect size was medium at 0.65.

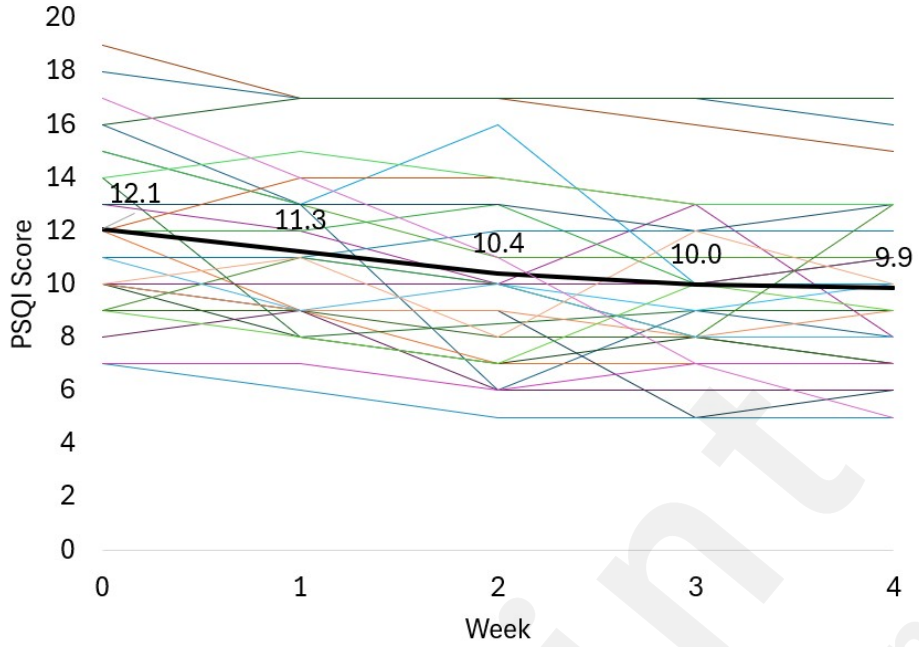
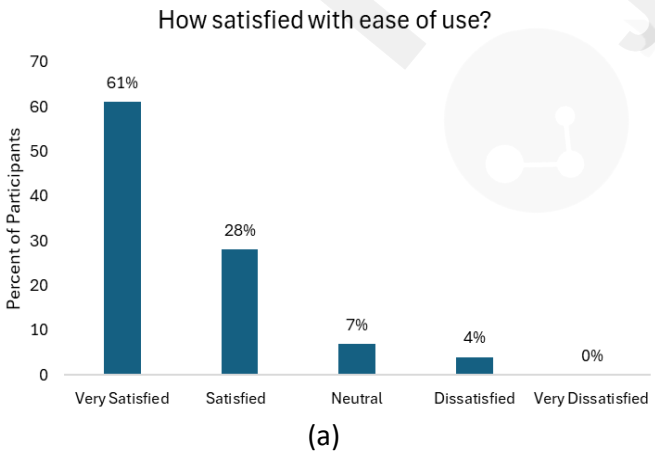


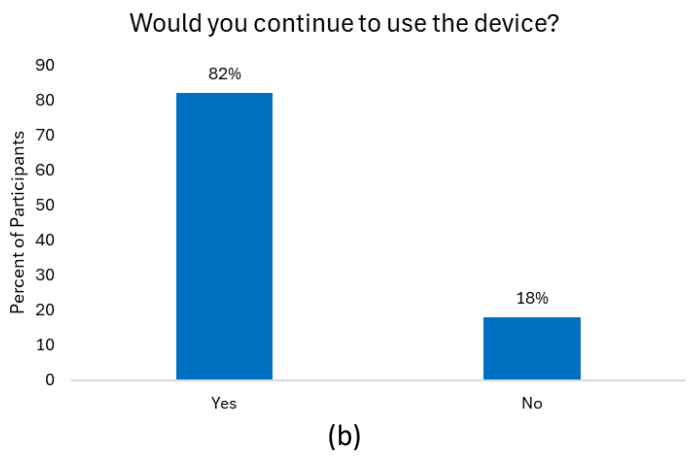
Figure 10. *The progression of PSQI scores through four weeks of treatment with ZenBud. The thin lines represent each individual participant, the thick line represents the group mean.*

Satisfaction and Acceptability

After the final treatment and assessment battery participants completed an exit survey asking questions regarding satisfaction with the treatment, acceptability, and quality of life impact. When asked about satisfaction with ease of use 89.3% of participants responded with very satisfied or satisfied (**Figure 11a**). Additionally, 82.1% reported that they would continue using the device if offered the opportunity (**Figure 11b**). When asked if the treatment was worth the time invested in the trial, 82.1% strongly agreed or agreed that the time invested was worth it (**Figure 11d**). When asked about the impact on quality of life, 78.6% of participants reported that the treatment somewhat or greatly impacted their quality of life (**Figure 11e**). When asked how long it took to feel initial effects, 53.6% of participants noticed effects in less than one week and 32.1% felt initial effects by one week (**Figure 11c**). When asked if they would recommend the treatment to someone with a similar condition, 75.0% of participants responded with very likely or likely (**Figure 11f**).



(a)



(b)

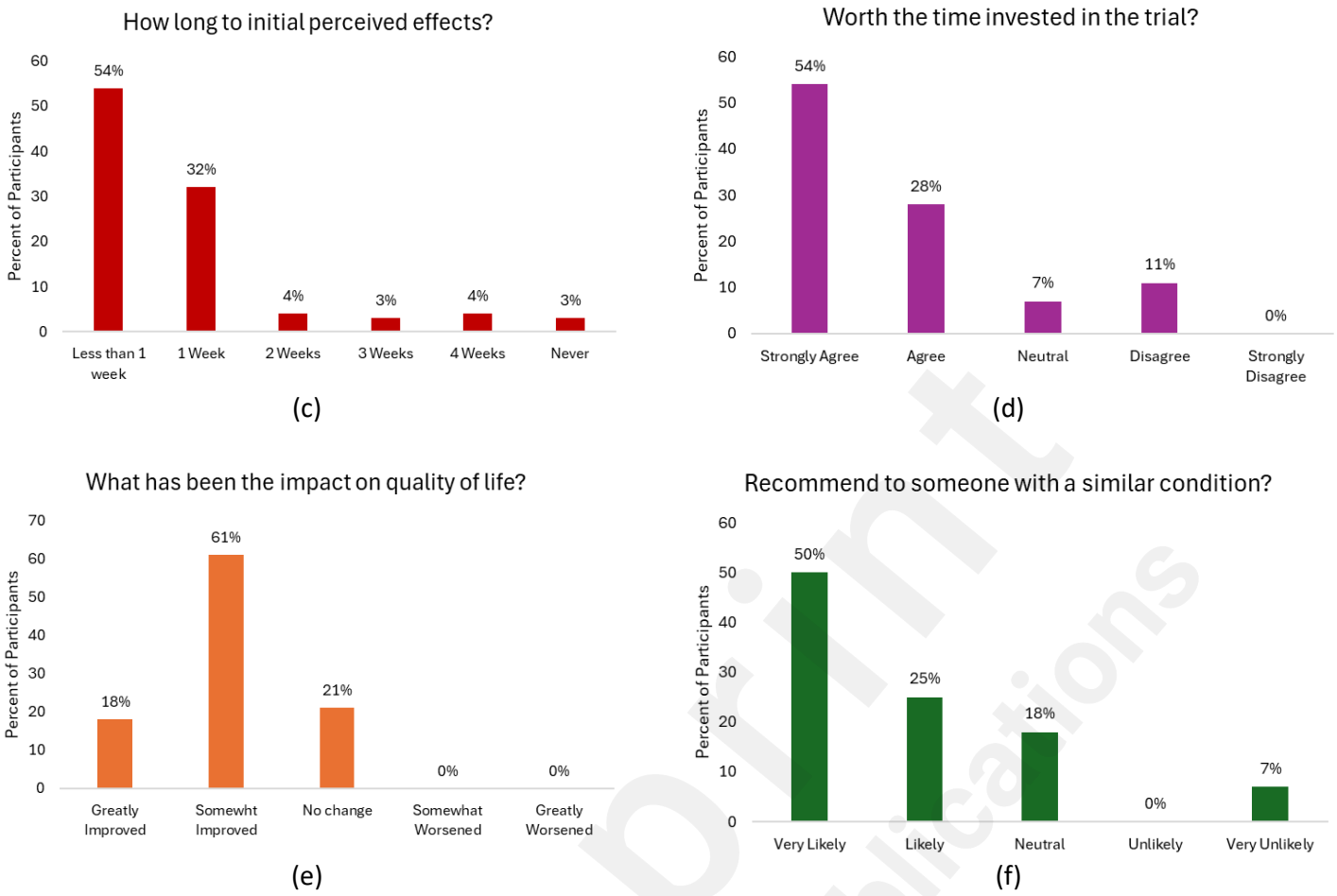


Figure 11. *Results of the exit survey. (a) Responses of the participants when asked "How satisfied were you with the ease of using the device?" (b) Responses of the participants when asked "Would you continue using this device for treatment?" (c) Responses of the participants when asked "How quickly did you feel the effects of the ZenBud device during your trial?" (d) Responses of the participants when asked "Do you feel the device was worth the time invested in the trial?" (e) Responses of the participants when asked "How did the device impact your overall quality of life?" (f) Responses of the participants when asked " How likely are you to recommend this device to others with similar conditions?"*

Adverse Events

Only one adverse event was reported throughout the duration of the trial. On the exit survey following completion of the four weeks of treatment, one participant reported the treatment would make them feel jittery for a short period of time afterwards. This effect was short-lived and classified as a mild AE that was probably device related. The participant reported that this side effect was not enough of an effect to make them stop treatment or drop out of the study. Overall, the high satisfaction rates as described above combined with the low rate of AE supports a strong benefit-to-risk profile for the ZenBud, however this study was done with a small sample size and these results need to be further validated with a larger sample size.

Discussion

The main objective of this study was to provide preliminary evidence of the efficacy, safety, and usability of the ZenBud for treating symptoms of anxiety in humans. Overall, the study represents one of the first clinical trials supporting the safety, patient tolerability, and efficacy of using low intensity focused ultrasound to the auricular branch of the vagus nerve for the treatment of anxiety symptoms.

Among the 28 participants, 92.9% demonstrated improvements in anxiety symptoms; 89.3% demonstrated improvements in depression symptoms; 82.1% demonstrated a reduction in symptoms of PTSD; and 65.5% demonstrated improvements in sleep quality after four weeks of treatment. The average score reduction on the BAI was clinically meaningful at 14.9 points (SD=10.6, p<.001, two-tailed dependent t-test), reflecting a general movement from severe anxiety symptoms to mild (Beck et al, 1988; Beck & Steer, 1990). The average score reduction on the BDI was clinically meaningful at 10.3 points (SD=7.8, p<.001, two-tailed dependent t-test), which was a 42.6% decrease in score, far greater than the 17% clinically meaningful threshold (Button et al., 2015). The average score reduction on the PCL-5 was clinically meaningful at 20.0 points (SD=20.5, p<.001, two-tailed dependent t-test) (Blanchard et al., 2023). It is also noteworthy to mention that the PCL-5 is commonly used to determine if an individual meets a provisional diagnosis of PTSD and requires further assessment to confirm the diagnosis. The cutoff score for meeting the criteria for a provisional PTSD diagnosis is 31-33. Based on using a cutoff score of 32, at the start of the study eighteen participants exceeded the threshold score for a provisional PTSD diagnosis. Upon completion of the study, fourteen of these participants (77.8%) had dropped their score below the threshold score of 32 and no longer met the requirements for a provisional PTSD diagnosis. The average score reduction on the PSQI was 2.2 (SD=3.1, p=.001, two-tailed dependent t-test) which while statistically significant, was not clinically meaningful, indicating that the improvements in anxiety, depression, and PTSD symptoms did not carry over into improved sleep quality. The effect sizes were also large for the BAI (Cohen's *d* = 1.06), BDI (Cohen's *d* = 0.81), and PCL-5 (Cohen's *d* = 0.94) indicating that the observed score improvements were substantial enough to have a meaningful impact beyond just statistical significance.

The extent of improvement in anxiety, depression, and PTSD observed in this study is comparable to the clinically meaningful results reported in other clinical trials featuring non-invasive vagus nerve stimulation as a treatment intervention. Srinivasan et al. (2024) conducted a randomized trial of transauricular vagus nerve stimulation (taVNS) with sixty retired school teachers who had been diagnosed with anxiety during the COVID-19 pandemic. . The participants did 30-minute sessions four times per week (16 total sessions) and demonstrated significantly greater reductions in Generalized Anxiety Disorder-7 (GAD-7) scores and salivary cortisol levels compared to control-group participants.. Zhang et al. (2024) investigated the effect of taVNS on anxiety symptoms and neural functioning in thirty individuals with Parkinson's disease and anxiety compared with thirty controls without anxiety. They treated Parkinson's patients with taVNS for two weeks and measured progress using the Hamilton Anxiety Rating Scale (HAM-A) and nerve activation in the bilateral prefrontal cortex during a verbal fluency task. After two weeks of taVNS treatment, the group demonstrated a significant decrease in HAM-A scores (*p*<.001) and increased activation of the left triangle portion of the inferior frontal gyrus. Ferreira et al. (2023) treated college students with chronic anxiety with a week of taVNS. Immediately post intervention and two weeks post intervention the students demonstrated substantial reductions in pain perception, Beck's Anxiety inventory scores, and masseter activation. Rong et al. (2017) treated 91 patients with mild to moderate depression with transcutaneous auricular vagus nerve stimulation for 30 minutes twice a day for 12 weeks. Upon completion of treatment the average reduction in score in the 24-item Hamilton Depression Rating Scale (HAM-D-24) was both statistically significant and clinically meaningful, the responder rate was 80%, and the remission rate was 39%. In our study, we saw similar results in only four weeks, making an investigation into longer treatment periods with LIFU an important area of future research.

The results of this study are also consistent with the results of studies investigating the use of transcranial focused ultrasound (tfUS) targeting the amygdala for the treatment of generalized anxiety disorders. Mahdavi et al. (2023) recruited twenty-five participants with treatment-refractory generalized anxiety disorder and treated them with tfUS targeting the right amygdala for eight weekly 10 minute sessions. The results showed an average reduction in BAI score of 12.88 +/- 10.42 points and an average reduction in HAM-A scores of -12.64 +/- 12.51. Chou et al. (2023) recruited thirty healthy individuals and compared activation of the amygdala, hippocampus, and dorsal anterior cingulate cortex during a fear task after treating them with active or sham tfUS targeting the left amygdala. They found decreased activation of the amygdala (*p*=.04),

hippocampus (p=.05), and dorsal anterior cingulate (p=.02) in the active tfUS group when compared to the sham. They also found decreased amygdala-insula (p=.03) and amygdala-hippocampal (p=.01) resting state functional connectivity and increased amygdala-ventromedial prefrontal cortex (p=.05) resting state functional connectivity.

While the results of this study are optimistic, this study was preliminary and suffers from several limitations. This study did not feature a control group, making it impossible to quantify the possible impact of a placebo effect. Other than participant reports, there was also no objective way of determining the exact amount of time the device was used by each participant. While the majority of participants were receiving no treatment during the study, there was no control over concurrent therapeutic modalities participants were receiving. Further research with larger sample sizes, control groups, control over concurrent treatment modalities, and physiological measurements need to be done to validate these findings and further negate the possibility of placebo effects.

Conclusion

This preliminary study provided justification for further research into the efficacy, safety, and feasibility of using LIFU to modulate the auricular branch of the vagus nerve and reduce the symptoms of anxiety, depression, and PTSD. Given the wide prevalence of anxiety disorders, depression, and PTSD, and the shortfalls of current treatment options, this novel treatment approach has potential to meaningfully improve patient outcomes and continued research is warranted.

References

Baek, H., Pahk, K. J., & Kim, H. (2017). A review of low-intensity focused ultrasound for neuromodulation. *Biomedical Engineering Letters*, *7*(2), 135–142. https://doi.org/10.1007/s13534-016-0007-y

Badran, B. W., Huffman, S. M., Dancy, M., Austelle, C. W., Bikson, M., Kautz, S. A., & George, M. S. (2022). A pilot randomized controlled trial of supervised, at-home, self-administered transcutaneous auricular vagus nerve stimulation (taVNS) to manage long COVID symptoms. *Research Square*, rs.3.rs-1716096. https://doi.org/10.21203/rs.3.rs-1716096/v1

Bardhoshi, G., Duncan, K., & Erford, B. T. (2016). Psychometric meta-analysis of the English version of the Beck Anxiety Inventory. *Journal of Counseling & Development*, *94*(3), 356-373. https://doi.org/10.1002/jcad.12090

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology*, *56*(6), 893–897. https://doi.org/10.1037//0022-006x.56.6.893

Beck, A. T., & Steer, R. A. (1990). *Manual for the Beck Anxiety Inventory.* San Antonio, TX, Psychological Corporation.

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. F. (1996). Comparison of Beck Depression Inventories-IA and-II in psychiatric outpatients. *Journal of Personality Assessment*, *67*(3), 588-597. https://doi.org/10.1207/s15327752jpa6703_13

Beck, A. T., Steer, R. A., & Carbin, M. G. (1988). Psychometric properties of the Beck Depression Inventory:

Twenty-five years of evaluation. *Clinical Psychology Review*, *8*(1), 77-100.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry*, *4*, 561–571. https://doi.org/10.1001/archpsyc.1961.01710120031004

Bentley, K. H., Bernstein, E. E., Wallace, B., & Mischoulon, D. (2021). Treatment for anxiety and comorbid depressive disorders: Transdiagnostic cognitive-behavioral strategies. *Psychiatric Annals*, *51*(5), 226–230. https://doi.org/10.3928/00485713-20210414-01

Blanchard, B. E., Johnson, M., Campbell, S. B., Reed, D. E., Chen, S., Heagerty, P. J., Marx, B.P., Kaysen, D. & Fortney, J. C. (2023). Minimal important difference metrics and test–retest reliability of the PTSD Checklist for DSM-5 with a primary care sample. *Journal of Traumatic Stress*, *36*(6), 1102-1114. https://doi.org/10.1002/jts.22975

Blevins, C. A., Weathers, F. W., Davis, M. T., Witte, T. K., & Domino, J. L. (2015). The Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5): Development and initial psychometric evaluation. *Journal of Traumatic Stress*, *28*(6), 489–498. https://doi.org/10.1002/jts.22059

Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Frontiers in Psychiatry*, *9*, 44. https://doi.org/10.3389/fpsyt.2018.00044

Bremner, J., Gurel, N., Wittbrodt, M., Nye, J., Alam, Z., Herring, I., ... & Inan, O. (2019). Non-invasive vagal nerve stimulation paired with stress exposure in posttraumatic stress disorder (PTSD). *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *12*(2), 438. https://doi.org/10.1016/j.brs.2018.12.417

Butt, M. F., Albusoda, A., Farmer, A. D., & Aziz, Q. (2020). The anatomical basis for transcutaneous auricular vagus nerve stimulation. *Journal of Anatomy*, *236*(4), 588-611. https://doi.org/10.1111/joa.13122

Button, K. S., Kounali, D., Thomas, L., Wiles, N. J., Peters, T. J., Welton, N. J., Ades, A. E., & Lewis, G. (2015). Minimal clinically important difference on the Beck Depression Inventory--II according to the patient's perspective. *Psychological Medicine*, *45*(15), 3269–3279. https://doi.org/10.1017/S0033291715001270

Buysse, D. J., Reynolds III, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research*, *28*(2), 193-213. https://doi.org/10.1016/0165-1781(89)90047-4

Chand S.P. & Marwaha R. Anxiety. [Updated 2023 Apr 24]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470361/

Chi, D., Zhang, Y., Zhou, D., Xu, G., & Bian, G. (2022). The effectiveness and associated factors of online psychotherapy on COVID-19 related distress: A systematic review and meta-analysis. *Frontiers in Psychology*, *13*, 1045400. https://doi.org/10.3389/fpsyg.2022.1045400

Chou, T., Deckersbach, T., Guerin, B., Sretavan Wong, K., Borron, B. M., Kanabar, A., Hayden, A. N., Long, M. P., Daneshzand, M., Pace-Schott, E. F., & Dougherty, D. D. (2024). Transcranial focused ultrasound of the amygdala modulates fear network activation and connectivity. *Brain Stimulation*, *17*(2), 312–320. https://doi.org/10.1016/j.brs.2024.03.004

Collins, M. N., & Mesce, K. A. (2022). A review of the bioeffects of low-intensity focused ultrasound and the benefits of a cellular approach. *Frontiers in Physiology*, *13*, 1047324. https://doi.org/10.3389/fphys.2022.1047324

Cotero, V., Fan, Y., Tsaava, T., Kressel, A. M., Hancu, I., Fitzgerald, P., Wallace, K., Kaanumalle, S., Graf, J., Rigby, W., Kao, T. J., Roberts, J., Bhushan, C., Joel, S., Coleman, T. R., Zanos, S., Tracey, K. J., Ashe, J., Chavan, S. S., & Puleo, C. (2019). Noninvasive sub-organ ultrasound stimulation for targeted neuromodulation. *Nature Communications*, *10*(1), 952. https://doi.org/10.1038/s41467-019-08750-9

Cox, R. C., & Olatunji, B. O. (2016). A systematic review of sleep disturbance in anxiety and related disorders. *Journal of Anxiety Disorders*, *37*, 104-129. https://doi.org/10.1016/j.janxdis.2015.12.001

Fabbri, M., Beracci, A., Martoni, M., Meneo, D., Tonetti, L., & Natale, V. (2021). Measuring subjective sleep quality: a review. *International Journal of Environmental Research and Public Health*, *18*(3), 1082. https://doi.org/10.3390/ijerph18031082

Feng, B., Chen, L., & Ilham, S. J. (2019). A review on ultrasonic neuromodulation of the peripheral nervous system: enhanced or suppressed activities?. *Applied Sciences*, *9*(8), 1637. https://doi.org/10.3390/app9081637

Ferreira, L. M., Brites, R., Fraião, G., Pereira, G., Fernandes, H., de Brito, J. A., Pereira Generoso, L., Maziero Capello, M.G., Pereira, G.S., Scoz, R.D., & Silva, M. (2023). Transcutaneous auricular vagus nerve stimulation modulates masseter muscle activity, pain perception, and anxiety levels in university students: A double-blind, randomized, controlled clinical trial. *Frontiers in Integrative Neuroscience*, 18, 1422312. https://doi.org/10.3389/fnint.2024.1422312

Fischer, R., Bortolini, T., Karl, J. A., Zilberberg, M., Robinson, K., Rabelo, A., Gemal, L., Wegerhoff, D., Nguyễn, T. B. T., Irving, B., Chrystal, M., & Mattos, P. (2020). Rapid review and meta-meta-analysis of self-guided interventions to address anxiety, depression, and stress during COVID-19 social distancing. *Frontiers in Psychology*, *11*, 563876. https://doi.org/10.3389/fpsyg.2020.563876

Forkus, S. R., Raudales, A. M., Rafiuddin, H. S., Weiss, N. H., Messman, B. A., & Contractor, A. A. (2023). The Posttraumatic Stress Disorder (PTSD) Checklist for DSM-5: A systematic review of existing psychometric evidence. *Clinical Psychology*, *30*(1), 110–121. https://doi.org/10.1037/cps0000111

Fydrich, T., Dowdall, D., & Chambless, D. L. (1992). Reliability and validity of the Beck Anxiety Inventory. *Journal of Anxiety Disorders*, *6*(1), 55-61. https://doi.org/10.1016/0887-6185(92)90026-4

Garakani, A., Murrough, J. W., Freire, R. C., Thom, R. P., Larkin, K., Buono, F. D., & Iosifescu, D. V. (2020). Pharmacotherapy of anxiety disorders: Current and emerging treatment options. *Frontiers in Psychiatry*, *11*, 595584. https://doi.org/10.3389/fpsyt.2020.595584

George, M. S., Ward, H. E., Jr, Ninan, P. T., Pollack, M., Nahas, Z., Anderson, B., Kose, S., Howland, R. H., Goodman, W. K., & Ballenger, J. C. (2008). A pilot study of vagus nerve stimulation (VNS) for treatment-resistant anxiety disorders. *Brain Stimulation*, *1*(2), 112–121. https://doi.org/10.1016/j.brs.2008.02.001

Gurel, N. Z., Wittbrodt, M. T., Jung, H., Shandhi, M. M. H., Driggers, E. G., Ladd, S. L., Huang, M., Ko, Y. A., Shallenberger, L., Beckwith, J., Nye, J. A., Pearce, B. D., Vaccarino, V., Shah, A. J., Inan, O. T., & Bremner, J. D. (2020). Transcutaneous cervical vagal nerve stimulation reduces sympathetic responses to stress in posttraumatic stress disorder: A double-blind, randomized, sham controlled trial. *Neurobiology of Stress*, *13*, 100264. https://doi.org/10.1016/j.ynstr.2020.100264

Haller, H., Breilmann, P., Schröter, M., Dobos, G., & Cramer, H. (2021). A systematic review and meta-analysis of acceptance- and mindfulness-based interventions for DSM-5 anxiety disorders. *Scientific Reports*, *11*(1), 20385. https://doi.org/10.1038/s41598-021-99882-w

Holwerda, S. W., Luehrs, R. E., Gremaud, A. L., Wooldridge, N. A., Stroud, A. K., Fiedorowicz, J. G., Abboud, F.M. & Pierce, G. L. (2018). Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety. *Journal of Neurophysiology*, *120*(1), 11-22. https://doi.org/10.1152/jn.00064.2018

Johnson, R. L., & Wilson, C. G. (2018). A review of vagus nerve stimulation as a therapeutic intervention. *Journal of inflammation research*, *11*, 203–213. https://doi.org/10.2147/JIR.S163248

Juan, E. J., González, R., Albors, G., Ward, M. P., & Irazoqui, P. (2014). Vagus nerve modulation using focused pulsed ultrasound: Potential applications and preliminary observations in a rat. *International Journal of Imaging Systems and Technology*, *24*(1), 67–71. https://doi.org/10.1002/ima.22080

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., Kiss, A., Podesser, B., Cassara, A. M., Tanghe, E., Samoudi, A. M., Tarnaud, T., Joseph, W., Marozas, V., Lukosevicius, A., Ištuk, N., Šarolić, A., Lechner, S., Klonowski, W., Varoneckas, G., … Széles, J. C. (2019). Current directions in the auricular vagus nerve stimulation I - A physiological perspective. *Frontiers in Neuroscience*, *13*, 854. https://doi.org/10.3389/fnins.2019.00854

KP, S., Joseph, N., & Sreenivasan, G. K. (2021). Sleep quality and anxiety level among college students. *International Journal of Research and Review*, *8*(10), 271-276. https://doi.org/10.52403/ijrr.20211036

Lamb, D. G., Porges, E. C., Lewis, G. F., & Williamson, J. B. (2017). Non-invasive vagal nerve stimulation effects on hyperarousal and autonomic state in patients with posttraumatic stress disorder and history of mild traumatic brain injury: Preliminary evidence. *Frontiers in Medicine*, *4*, 124. https://doi.org/10.3389/fmed.2017.00124

Mahdavi, K. D., Jordan, S. E., Jordan, K. G., Rindner, E. S., Haroon, J. M., Habelhah, B., Becerra, S. A., Surya, J. R., Venkatraman, V., Zielinski, M. A., Spivak, N. M., Bystritsky, A., & Kuhn, T. P. (2023). A pilot study of low-intensity focused ultrasound for treatment-resistant generalized anxiety disorder. *Journal of Psychiatric Research*, *168*, 125–132. https://doi.org/10.1016/j.jpsychires.2023.10.039

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S. G., & Russell, J. (2007). Neurobiology of depression: An integrated view of key findings. *International Journal of Clinical Practice*, *61*(12), 2030–2040. https://doi.org/10.1111/j.1742-1241.2007.01602.x

Martin, E. I., Ressler, K. J., Binder, E., & Nemeroff, C. B. (2009). The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. *The Psychiatric Clinics of North America*, *32*(3), 549–575. https://doi.org/10.1016/j.psc.2009.05.004

Moghimi, E., Stephenson, C., Agarwal, A., Nikjoo, N., Malakouti, N., Layzell, G., O'Riordan, A., Jagayat, J., Shirazi, A., Gutierrez, G., Khan, F., Patel, C., Yang, M., Omrani, M., & Alavi, N. (2023). Efficacy of an electronic cognitive behavioral therapy program delivered via the online psychotherapy tool for depression and anxiety related to the COVID-19 pandemic: Pre-post pilot study. *JMIR Mental Health*, *10*, e51102. https://doi.org/10.2196/51102

Mollayeva, T., Thurairajah, P., Burton, K., Mollayeva, S., Shapiro, C. M., & Colantonio, A. (2016). The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis. *Sleep Medicine Reviews*, *25*, 52-73. https://doi.org/10.1016/j.smrv.2015.01.009

Noble, L. J., Gonzalez, I. J., Meruva, V. B., Callahan, K. A., Belfort, B. D., Ramanathan, K. R., Meyers, E., Kilgard, M. P., Rennaker, R. L., & McIntyre, C. K. (2017). Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats. *Translational Psychiatry*, *7*(8), e1217. https://doi.org/10.1038/tp.2017.191

Penninx, B. W., Pine, D. S., Holmes, E. A., & Reif, A. (2021). Anxiety disorders. *Lancet*, 397(10277), 914–927. https://doi.org/10.1016/S0140-6736(21)00359-7

Perrotta, G. (2021). Maladaptive stress: Theoretical, neurobiological and clinical profiles. *Archives of Depression and Anxiety*, *7*(1), 001-007.

Riis, T., & Kubanek, J. (2022). Effective ultrasonic stimulation in human peripheral nervous system. *IEEE Transactions on Bio-Medical Engineering*, *69*(1), 15–22. https://doi.org/10.1109/TBME.2021.3085170

Rong, P., Liu, J., Wang, L., Liu, R., Fang, J., Zhao, J., Zhao, Y., Wang, H., Vangel, M., Sun, S., Ben, H., Park, J., Li, S., Meng, H., Zhu, B., & Kong, J. (2016). Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. *Journal of Affective Disorders*, *195*, 172–179. https://doi.org/10.1016/j.jad.2016.02.031

Srinivasan, V., Ruthuvalan, V., Raja, S., Jayaraj, V., Sridhar, S., Kothandaraman, M., Suganthirababu, P., Abathsagayam, K., Vishnuram, S., Alagesan, J., & Vasanthi, R. K. (2024). Efficacy of Vagal nerve stimulation on anxiety among elderly retired teachers during COVID-19 pandemic. *Work*, (Preprint), 1-8. https://doi.org/10.3233/WOR-230356

Stockings, E. A., Degenhardt, L., Dobbins, T., Lee, Y. Y., Erskine, H. E., Whiteford, H. A., & Patton, G. (2016). Preventing depression and anxiety in young people: a review of the joint efficacy of universal, selective and indicated prevention. *Psychological medicine*, *46*(1), 11–26. https://doi.org/10.1017/S0033291715001725

Ströhle, A., Gensichen, J., & Domschke, K. (2018). The diagnosis and treatment of anxiety disorders. *Deutsches Arzteblatt International*, 155(37), 611–620. https://doi.org/10.3238/arztebl.2018.0611

Tasker, J. G., & Joëls, M. (2015). The synaptic physiology of the central nervous system response to stress. *Neuroendocrinology of Stress*, 43-70. https://doi.org/10.1002/9781118921692.ch3

Wang, Y. P., & Gorenstein, C. (2013). Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. *Brazilian Journal of Psychiatry*, *35*, 416-431. https://doi.org/10.1590/1516-4446-2012-1048

Williamson, J. B., Jaffee, M. S., & Jorge, R. E. (2021). Posttraumatic stress disorder and anxiety-related conditions. *Continuum*, *27*(6), 1738–1763. https://doi.org/10.1212/CON.0000000000001054

Wittbrodt, M. T., Gurel, N. Z., Nye, J. A., Shandhi, M. M. H., Gazi, A. H., Shah, A. J., Pearce, B. D., Murrah, N., Ko, Y. A., Shallenberger, L. H., Vaccarino, V., Inan, O. T., & Bremner, J. D. (2021). Noninvasive cervical vagal nerve stimulation alters brain activity during traumatic stress in individuals with posttraumatic stress disorder. *Psychosomatic Medicine*, 83(9), 969–977. https://doi.org/10.1097/PSY.0000000000000987

Zhang, H., Shan, A. D., Wan, C. H., Cao, X. Y., Yuan, Y. S., Ye, S. Y., Gao, M. X., Gao, L. Z., Tong, Q., Gan, C. T.,

Sun, H. M., & Zhang, K. Z. (2024). Transcutaneous auricular vagus nerve stimulation improves anxiety symptoms and cortical activity during verbal fluency task in Parkinson's disease with anxiety. *Journal of Affective Disorders*, 361, 556–563. https://doi.org/10.1016/j.jad.2024.06.083

Supplementary Files

Multimedia Appendixes

Zenbud user manual.
URL: http://asset.jmir.pub/assets/67c9074f7fba6b797fea132ef63e5eb7.pdf