Journal of Visualized Experiments

Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58984R1
Full Title:	Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans
Keywords:	tVNS, taVNS, vns, Vagus Nerve, Auricular Vagus, Transcutaneous auricular vagus nerve stimulation, ear stimulation
Corresponding Author:	Bashar W Badran City College of New York New York, NY UNITED STATES
Corresponding Author's Institution:	City College of New York
Corresponding Author E-Mail:	basharwbadran@gmail.com
Order of Authors:	Bashar W Badran
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Open Access (US$4,200)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	New York City

TITLE:

Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans

AUTHORS & AFFILIATIONS:

*Bashar W. Badran[1,2,3], *Alfred B. Yu[2], Devin Adair[1], Georgia Mappin[3], William H. DeVries[3], Dorothea D. Jenkins[4], Mark S. George[3,5,6], Marom S. Bikson[1]

[1] Department of Biomedical Engineering, City College of New York, New York, NY
[2] U.S. Army Research Laboratory, Aberdeen Proving Ground, MD
[3] Brain Stimulation Laboratory, Department of Psychiatry, Medical University of South Carolina, Charleston SC
[4]Department of Pediatrics, Medical University of South Carolina, Charleston SC
[5] Department of Neurology, Medical University of South Carolina, Charleston SC
[6] Ralph H. Johnson VA Medical Center, Charleston SC

*Indicates Co-First Authorship

Alfred B. Yu: alfred.b.yu.civ@mail.mil
Devin Adair: devinomega@gmail.com
Georgia Mappin: mappin@musc.edu
William H. DeVries: devriesw@musc.edu
Dorothea D. Jenkins: jenkd@musc.edu
Mark S. George: georgem@musc.edu
Marom S. Bikson: bikson@ccny.cuny.edu

Corresponding Author:
Bashar W. Badran, Ph.D.
basharwbadran@gmail.com
843-792-1006

KEYWORDS:

taVNS, tVNS, VNS, Transcutaneous Auricular Vagus Nerve Stimulation, vagus nerve stimulation, ear stimulation

SUMMARY:

A methodological description of the technique, potential targets, and proper administration of transcutaneous auricular vagus nerve stimulation (taVNS) on the human ear is described.

ABSTRACT:

Non-invasive vagus nerve stimulation (VNS) may be administered *via* a novel, emerging neuromodulatory technique known as transcutaneous auricular vagus nerve stimulation (taVNS). Unlike cervically-implanted VNS, taVNS is an inexpensive and non-surgical method used to modulate the vagus system. taVNS is appealing as it allows for rapid translation of basic VNS research and serves as a safe, inexpensive, and portable neurostimulation system for the future

treatment of central and peripheral disease. The background and rationale for taVNS is described, along with electrical and parametric considerations, proper ear targeting and attachment of stimulation electrodes, individual dosing *via* determination of perception threshold (PT), and safe administration of taVNS.

INTRODUCTION:

Cranial nerve X, better known as the vagus nerve, is a large nerve tract that originates in the brainstem of the central nervous system and travels throughout the periphery, targeting every major organ in the thorax and abdomen (**Figure 1**)[1]. Vagus nerve stimulation (VNS) involves surgical implantation of bipolar electrodes around the left cervical branch of the vagus nerve. Electrical pulses are delivered to the vagus nerve *via* an implanted pulse generator (IPG) surgically implanted in the chest[2]. Although VNS is currently FDA-approved for epilepsy, refractory depression, and chronic obesity, it is a costly procedure requiring a hospital visit and surgery. Long-term safety of VNS is well established, and the majority of safety considerations regard current intensity related side effects (hoarse voice, throat pain) without serious stimulation-related adverse effects over the past 25 years of its clinical use[3].

Recently, a noninvasive form of VNS known as transcutaneous auricular vagus nerve stimulation (taVNS) has emerged[4]. taVNS delivers electrical stimulation to the auricular branch of the vagus nerve (ABVN), an easily accessible target that innervates the human ear[5]. Over the last decade, several groups have demonstrated the safety and tolerability of this method[6-8], including central and peripheral nervous system effects[9,10], and behavioral effects[7,11-13] in neuropsychiatric populations. taVNS is also being explored in individuals as a promising enhancer of cognitive[14,15] and social functioning[16-18]. As taVNS is becoming established, it offers the ability for researchers and clinicians to rapidly translate the promising VNS research that has been described in various disorders ranging from neurological and psychological trauma[19-21], addiction[22], inflammation[23], and tinnitus[24,25].

In principle, taVNS is methodologically similar to conventionally administered transcutaneous electrical nerve stimulation (TENS) used to treat musculoskeletal pain disorders[26]. The difference is that taVNS is delivered to specific anatomical ear targets that are believed to be innervated by the ABVN[5]. The field is still determining optimal stimulation targets[27], although the two most common placements are the anterior wall of the outer ear canal (tragus) and the cymba conchae. Sham stimulation may be conducted by stimulating the earlobe of the ear, an area believed to have minimal ABVN innervation (**Figure 2**)**.** Alternatively, sham may be delivered via a passive control method in which electrodes are attached to active sites, but no stimulation is delivered. Stimulation parameters may vary between groups, however according to the literature, stimulation is delivered in a pulsatile fashion (pulse width: 250-500 μs, frequency: 10-25 Hz) and delivered at an individualized constant current (<5 mA). Stimulation current varies by individual and experimental protocol, with many groups exploring various intensities as a function of an individual perceptual threshold (PT). The PT is defined as the minimum amount of current eliciting a perceived sensation at the target site and is usually determined *via* parametric estimation by customized sequential testing (PEST) software described in this report.

taVNS is a safe technique that may be administered in the laboratory or clinical setting. Side-effects of taVNS are minimal, with skin irritation or redness being the most common side-effect. Most taVNS studies explore stimulation of the left ear, as it is believed to be safer, although data in a large trial (Badran *et al*. 2018) reveal that right-sided stimulation has no increases in the risk of adverse events. Due to the wealth of literature in unilateral-left side stimulation, we will illustrate the typical taVNS set-up for laboratory studies investigating the use of left-sided taVNS as an intervention.

PROTOCOL:

This experimental protocol illustrates a typical taVNS set-up for use in a laboratory or clinical setting in which we target stimulating the anterior wall of the auditory canal (tragus) in a supine posture with an 8mm diameter round metal electrode. These methods can be mimicked for alternative active treatment sites by simply changing electrode position to the cymba concha. All methods and procedures have been IRB approved by the Human Research Protection Program (HRPP) at City College New York.

1. Materials

1.1. Ensure all materials required to administer taVNS are prepared (**Figure 3**). The taVNS stimulator may be either a battery driven device that meets local safety regulations or powered from a conventional electrical outlet with built-in safety mechanisms that prevent unintended electrical surges. A constant current (current controlled) stimulator with a maximum output of 5 mA is required.

1.2. For taVNS, use stimulation electrodes made of a round conductive metal (tin, Ag/AgCl, gold) combined with a conductive medium such as electrolyte gel or conductive paste (see table of materials). Alternatively, use conductive electrodes made with flexible conductive carbon electrodes and conductive gel that may or may not be adhesive. Never place electrodes directly on the skin without a conductive medium, as this may pose unnecessary risk to the participant and can cause discomfort or pain.

1.3. Use computer running script software (see **Table of Materials**) that is programmed and used to control the stimulator and initiate stimulation with specific parameters. These parameters include current intensity (mA), pulse width (μs), frequency (Hz), duty cycle (On/Off time, s), session duration (min).

1.4. Use alcohol preparation pads (70% isopropyl alcohol) to prepare the skin surface before attaching electrodes to the ear. This removes surface oils from the skin surface and reduces the resistance of the skin, ensuring stimulation is delivered at safe power levels.

2. Ear Targeting and Skin Preparation

2.1. Use the following general inclusion criteria for conducting taVNS in the research setting: Age 18-70, no facial or ear pain, no recent ear trauma, no metal implants including pacemakers, not pregnant.

2.2. In the experiments involving healthy participants in a laboratory setting, use the following exclusion criteria: personal or family history of seizure, mood, or cardiovascular disorders, dependence on alcohol or recent illicit drug use, on any pharmacological agents known to increase seizure risk.

2.3. Seat the participant on a comfortable bed or chair in a supine or other relaxed position with legs elevated and head supported.

2.4. Inspect the left ear of the participant. Ensure no jewelry is attached and all make-up and lotion are removed. Confirm there are no skin-related contraindications at the site of stimulation, including sun burn, cuts, lesions, open sores.

2.5. Find the stimulation target, landmarked by the anterior wall of the outer ear canal externally by finding the tragus. Stimulation will be delivered to the portion of the ear canal directly behind the tragus (**Figure 4**).

2.6. Use an alcohol prep pad to gently scrub the target site, both internally and externally, to decrease skin resistance and increase conductance.

3. Electrode Preparation and Placement

3.1. If using non-disposable electrodes, visually inspect electrodes to ensure clean, corrosion-free surface is exposed. Ensure that electrodes are disinfected to prevent spread of bacteria between subjects. This can be done using alcohol or sterilization wipes to scrub the electrodes. If using disposable electrodes, skip to step 3.2.

3.2. Spread a thin layer of conductive paste to the surface of the electrode evenly. This will distribute electricity to the stimulation site. For an 8 mm diameter round electrode, a pea-sized amount of paste is sufficient. Spread the paste using a narrow wooden applicator to form a thin layer <1 mm of paste on both electrodes.

3.3. Connect electrode cables to the stimulation device while the device is turned off and verify the polarity of the electrodes (red/positive electrode – anode, black/negative electrode - cathode). This is an important detail as targeting is polarity specific - the anode (red/positive terminal) is the electrode placed inside the ear canal and targeting the anterior wall of the outer ear canal. The cathode (black/negative terminal) sits on the outside of the ear attached to the tragus. For sham stimulation, the anode is placed on the anterior side of the ear.

3.4. Clip the spring electrode onto the tragus with the anode making contact with the anterior wall of the outer ear canal and the cathode contacting the anterior part of the tragus.

NOTE: If conducting sham stimulation, clip the electrode onto the earlobe (active control). Alternatively, sham stimulation may be delivered by attaching stimulation clips to active site and delivering no electrical current (passive control).

3.5. As subjects will feel the pressure of the electrodes clipped to their ear, ensure this pressure is not uncomfortable or disruptive to regional blood flow as demonstrated by pale white skin at clip site or physical pain sensed by the subject. After this point, determine perceptual threshold (PT) which will be described in the next procedural step.

4. Determination of Perceptual Threshold (PT)

NOTE: Perceptual threshold is a critical value used to determine the power of taVNS stimulation. This value is defined as the minimum amount of electricity required to perceive electrical stimulation on the skin described as a pricking or tingling sensation.

4.1. Determine the PT using a simple step-up and step-down binary parametric search. First turn on the stimulator and set the output to 3 mA. Deliver a 1 second train of taVNS stimulation at desired pulse width (typically 250 μs – 500 μs) and frequency (25 Hz, can vary based on application).

4.2. Ask the subject whether they felt the stimulation. Sensation is generally reported as a "tickle" or "pricking" sensation.

4.2.1. If YES, turn down stimulation intensity by 50% and repeat step 4.2. If NO, increase stimulation intensity by 50% and repeat step 4.2.

4.3. Repeat the process described in step 4.2 until recording a minimum of 4 "YES" responses in which the 4th YES response must come after a NO. The intensity (in mA) of the PT will the value at which the subject says their fourth YES response to.

4.4. Use the example PT threshold finding is listed in **Table 1** to assist in PT determination.

5. Delivering Stimulation

5.1. Once the subject is comfortable stimulation electrodes with properly attached to the desired target, and the perceptual threshold determined at the desired pulse width and frequency, begin the stimulation.

5.2. Use a computer running a pulse generating GUI (e.g., stimDesigner, freeware included with this manuscript) connected to a data acquisition unit (DAQ) to drive the stimulation system. The software should output TTL pulses as programmable settings (**Figure 5**). The TTL pulses will be sent via a BNC cable to the stimulator "trigger in" port. This interface software/stimulator

interface allows modulation of frequency, duty cycle (on/off time), and session duration (**Figure 6**). The GUI used is attached as a free, open-source resource with this manuscript.

5.2.1. Ensure that stimulation is delivered at super-threshold levels, such as 200% of PT[8,9]. For example, if the PT was determined to be 0.8 mA, stimulation will be delivered at 1.6 mA.

5.2.2. Ensure that the guidelines for duty cycles are followed when conducting long stimulation sessions. Typical duty cycles have 30-60 s "on" periods and 60-120 s "off" periods, or 20% -50% duty cycles.

5.2.3. Vary the length of stimulation session (total time). Studies suggest that 30-60 minute stimulation sessions at a 25% duty cycle is safe and free of any acute side effects or adverse events. These sessions can be repeated with 12-24 hours between sessions safely.

NOTE: taVNS safety is unclear for longer periods of stimulation sessions, larger percentage duty cycles (>40%), accelerated paradigms, and higher stimulation current doses.

6. After taVNS

6.1. When stimulation is completed, record objective data regarding the stimulation discomfort and side effects. Although taVNS, like implantable VNS, has limited safety concerns[8,28], monitor and record sensation, discomfort, and any adverse events on a rating from 0-10[29].

6.2. Remove the stimulation electrode from the ear and clean residual conductive paste from the subject's ear using an alcohol prep pad.

6.3. Use alcohol to clean and disinfect the stimulation electrode immediately upon removing from the subject's ear.

6.4. Inspect the ear for redness or irritation at the stimulation site and record any observations.

REPRESENTATIVE RESULTS:

When proper skin preparation is conducted, perceptual thresholds are inversely correlated with stimulation pulse width. As pulse width increases, the perceptual threshold decreases (**Figure 7**). Initial studies by this group exploring the effect of pulse width on PT in healthy individuals (meeting inclusion/exclusion criteria listed above), determined that the combined overall (n=15, 7 female, mean age 26.5 $\pm$ 4.99) PT at 100 μs = 3.92 $\pm$ 1.1 mA; 200 μs = 2.24 $\pm$ 0.74 mA; 500 μs = 1.24 $\pm$ 0.41 mA. These thresholds suggest that a constant current stimulator with capacity of delivering up to 5 mA of current is required for stimulation of 500 μs pulse width parameters, and a minimum of a 10mA stimulator is required for lower pulse widths (**Table 2**). Fine tuning of the current is required, with increments of 0.1 mA are necessary for precise stimulation.

Delivering stimulation at 200% PT is tolerable and relatively pain free as demonstrated by pain numerical rating scales (NRS) scales[9,30]. The NRS scale is a rating system for pain from 0-10 in which individuals report pain or discomfort[29]. Both Active and Sham stimulation rate similarly low pain levels (NRS <3 for all stimulation pulse widths. More specifically, the biologically-active pulse width of 500 μs delivered at 25 Hz is reported on average to rate as Active = 1.98 ± 0.83, Sham = 2.17 ± 1.27 (n=25, 9 female, mean age 25.16 ± 4.16 years) (**Table 3**). Pain ratings for other parameters are no more painful than the 25 Hz parameter and the details can be found in the groups' prior work[30].

Safety and tolerability of 30 min to 1 hour sessions at a 20-50% duty cycle has been widely reported in the literature with some studies delivering multiple sessions in the same day spread 12-15 hours apart[12,31]. No serious adverse events have been reported from 60 subjects participating in several series of experiments with subjects participating from 1 to 8 repeated visits spread a minimum of 24 hours apart.

taVNS, when administered as reported in this manuscript, has been demonstrated to modulate the autonomic nervous system, induce functional brain activity changes as measured by fMRI BOLD, and piloted to treat neuropsychiatric disorders and aid in rehabilitation.

FIGURE AND TABLE LEGENDS:

Figure 1: Vagus Nerve Efferent Projections and Cross-section. **A**. Efferent projections of the vagus nerve target every major organ on the body with wide effects on bodily function **B**. Cross-section of the vagus nerve, demonstrating the inside anatomy of the nerve as a series of bundles of nerves all contained within one major pathway.

Figure 2: taVNS Ear Targets. Targeting the ABVN can be accomplished by stimulating the anterior wall of the outer ear canal, landmarked notably by the tragus (A1), or cymba conchae (A2). Sham stimulation is administered to the earlobe (S).

Figure 3: Key Components. The minimum required components for proper administration of taVNS are the following A) ear stimulation electrodes, B) conductive gel and alcohol prep pads, C) computer capable of sending and receiving TTL pulses to a D) constant current stimulator to trigger stimulation.

Figure 4: Example Setup. This photo shows an individual receiving taVNS of the left ear while in position to undergo an experimental paradigm.

Figure 5. Screenshot of the GUI used for stimulation.

Figure 6: Electrical Stimulation Waveform Manipulations. Direct square wave electrical current can be delivered at various parameters. This figure demonstrates key properties of the waveform that can be changed in order to achieve desired biologic effects.

Figure 7: Perceptual Threshold Values at Increasing Pulse Widths. As pulse width increases, perceptual threshold (PT) decreases. Most healthy individuals will have a PT within 2 standard deviations (SD) of these mean values.

Table 1: Example of How to Determine Perceptual Threshold (PT). This table shows an example sequence of Yes/No responses used to parametrically determine PT.

Table 2: Stimulation Current Levels. Values of stimulation current in mA (200% PT) for each pulse width (n=15).

Table 3: PT, Stimulation Current, and Pain Values for Suggested Stimulation Parameters. Values of stimulation current in mA (200% PT) for each pulse width (n=25).

Supplemental File: Freeware GUI used in this protocol.

DISCUSSION:

As in all novel modalities, all the described steps are critical in the safe administration of taVNS. Of ultimate concern is subject safety, which includes not only mitigating risks before taVNS *via* proper screening, but also monitoring subjects during stimulation for discomfort, pain, or adverse events. Here are the three most important consideration for administering taVNS. Screening for taVNS contraindications – contraindications are as follows: any current or past history of cardiovascular disorders, facial or ear pain, recent ear trauma, metal implants above the level of the neck. For proper subject skin preparation, removing any surface oils, dirt, or makeup from the surface of the skin with alcohol helps with conductivity of the electrodes, reduces stimulation voltage required to drive the stimulator, and ultimately results in a more tolerable and safe stimulation session. It is encouraged to use a stimulator and electrodes meeting Low Output Transcranial Electrical Stimulation (LOTES) guidelines[32]. LOTES sets guidelines and industry standards for electrical stimulators that are built for stimulation of the head and neck and it is encouraged for groups to read this document before building their own systems. It is recommended to use either a FDA-cleared plug-in stimulator (see **Table of Materials**), or a low voltage (<50 V), battery powered, constant current stimulator with appropriate safety measures built-in to avoid unintended over-delivery of current to the stimulation site. Ensure that electrodes are manufactured and assembled for specific use in taVNS. Ensure that the current manufacturing and engineering guidelines are followed as a reference if lab-made customized systems are used.

One consideration for taVNS is to ensure that the voltage output of the constant current stimulator can surmount the resistance of the skin and deliver the current required for stimulation. Ohm's law (V=IR) demonstrates the relationship between current (I) and skin resistance (R). A minimum of a 20 V tabletop stimulator is recommended to avoid an underpowered system. Heat generated from the scalp or environment may degrade the conductive paste. If this occurs, it is recommended to stop stimulation and re-prep skin and electrodes with new conductive paste.

A limitation of taVNS is the vast parameter space. It is unknown as to which is more important – pulse width or frequency. There is a lacking data in recent taVNS trials that answer such questions. The various behavioral effects are derived from a variety of pulse widths, frequencies and stimulation currents[13,33-39].

At this time, it is suggested that the 500 µs pulse width to be the most biologically active[9]. With respect to frequency, it has been demonstrated that 25 Hz is an effective frequency, although current investigations into optimal such as higher frequencies (>25 Hz), bilateral stimulation (left and right ears), and investigational burst paradigms are being conducted. Studies exploring different parameters of stimulation, alternative stimulation sites, and duty cycle optimization are needed to advance and refine the taVNS method.

taVNS is a promising non-invasive alternative to conventional VNS. taVNS provides an inexpensive (<$5,000 in the demonstrated experimental setup, cost heavily dependent on type of stimulator used) and straightforward method that can be used to translate positive findings in animal models exploring the use of VNS on a variety of disorders, noninvasively modulate the autonomic nervous system, and potentially miniaturized and optimized for at-home neuromodulation for the treatment of neuropsychiatric and other disorders.

The future potential and possible applications of taVNS are vast. taVNS can serve as a promising adjunct or standalone treatment for neuropsychiatric disorders such as depression and epilepsy, taVNS-paired rehabilitation training to restore or accelerate learning of a behavior[40], decrease inflammatory response[41,42], and can potentially be used to enhance performance and autonomic function[8,10].

ACKNOWLEDGMENTS:

Research reported in this publication was supported by funding from the National Institutes of Health National Center of Neuromodulation for Rehabilitation, NIH/NICHD Grant Number P2CHD086844 which was awarded to the Medical University of South Carolina. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or NICHD.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1 Berthoud, H.R. & Neuhuber, W.L. Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience.* **85** (1-3), 1-17, doi:10.1016/S1566-0702(00)00215-0 (2000).

2 George, M.S. *et al.* Vagus nerve stimulation: a new form of therapeutic Brain Stimulation. *CNS Spectrums.* **5** (11), 43-52 (2000).

3 Nemeroff, C.B. *et al.* VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology.* **31** (7), 1345-1355, doi:10.1038/sj.npp.1301082 (2006).

4 Ventureyra, E.C. Transcutaneous vagus nerve stimulation for partial onset seizure

therapy. *Child's Nervous System.* **16** (2), 101-102 (2000).
5 Peuker, E.T. & Filler, T.J. The nerve supply of the human auricle. *Clinical Anatomy.* **15** (1), 35-37 (2002).
6 Kreuzer, P.M. *et al.* Transcutaneous vagus nerve stimulation: retrospective assessment of cardiac safety in a pilot study. *Frontiers in Psychiatry.* **3**, 70, doi:10.3389/fpsyt.2012.00070 (2012).
7 Kreuzer, P.M. *et al.* Feasibility, safety and efficacy of transcutaneous vagus nerve stimulation in chronic tinnitus: an open pilot study. *Brain Stimulation.* **7** (5), 740-747, doi:10.1016/j.brs.2014.05.003 (2014).
8 Badran, B.W. *et al.* Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimulation.* **11** (4), 699-708, doi:10.1016/j.brs.2018.04.004 (2018).
9 Badran, B.W. *et al.* Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. *Brain Stimulation.* **11** (3), 492-500, doi:10.1016/j.brs.2017.12.009 (2018).
10 Clancy, J.A. *et al.* Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. *Brain Stimulation.* **7** (6), 871-877, doi:10.1016/j.brs.2014.07.031 (2014).
11 Usichenko, T., Hacker, H., & Lotze, M. Transcutaneous auricular vagal nerve stimulation (taVNS) might be a mechanism behind the analgesic effects of auricular acupuncture. *Brain Stimulation.* **10** (6), 1042-1044, doi:10.1016/j.brs.2017.07.013 (2017).
12 Rong, P. *et al.* Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. *Journal of Affective Disorders.* **195**, 172-179, doi:10.1016/j.jad.2016.02.031 (2016).
13 Bauer, S. *et al.* Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimulation.* **9** (3), 356-363 (2016).
14 Jacobs, H.I., Riphagen, J.M., Razat, C.M., Wiese, S., & Sack, A.T. Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiology of Aging.* **36** (5), 1860-1867, doi:10.1016/j.neurobiolaging.2015.02.023 (2015).
15 Jongkees, B.J., Immink, M.A., Finisguerra, A., & Colzato, L.S. Transcutaneous Vagus Nerve Stimulation (tVNS) Enhances Response Selection During Sequential Action. *Frontiers in Psychology.* **9**, 1159, doi:10.3389/fpsyg.2018.01159 (2018).
16 Sellaro, R., de Gelder, B., Finisguerra, A., & Colzato, L.S. Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex.* **99**, 213-223, doi:10.1016/j.cortex.2017.11.007 (2018).
17 Jin, Y. & Kong, J. Transcutaneous vagus nerve stimulation: a promising method for treatment of autism spectrum disorders. *Frontiers in Neuroscience.* **10** (2016).
18 Colzato, L.S., Ritter, S.M., & Steenbergen, L. Transcutaneous vagus nerve stimulation (tVNS) enhances divergent thinking. *Neuropsychologia.* **111**, 72-76, doi:10.1016/j.neuropsychologia.2018.01.003 (2018).
19 George, M.S. *et al.* Vagus nerve stimulation for the treatment of depression and other neuropsychiatric disorders. *Expert Review of Neurotherapeutics.* **7** (1), 63-74, doi:10.1586/14737175.7.1.63 (2007).
20 Kong, J., Fang, J., Park, J., Li, S., & Rong, P. Treating Depression with Transcutaneous

Auricular Vagus Nerve Stimulation: State of the Art and Future Perspectives. *Frontiers in Psychiatry.* **9**, 20, doi:10.3389/fpsyt.2018.00020 (2018).

21 Dawson, J. *et al.* Safety, feasibility, and efficacy of vagus nerve stimulation paired with upper-limb rehabilitation after ischemic stroke. *Stroke.* **47** (1), 143-150 (2016).

22 Liu, H. *et al.* Vagus nerve stimulation inhibits heroin-seeking behavior induced by heroin priming or heroin-associated cues in rats. *Neuroscience Letters.* **494** (1), 70-74, doi:10.1016/j.neulet.2011.02.059 (2011).

23 Zhang, Y. *et al.* Chronic Vagus Nerve Stimulation Improves Autonomic Control and Attenuates Systemic Inflammation and Heart Failure Progression in a Canine High-Rate Pacing ModelCLINICAL PERSPECTIVE. *Circulation: Heart Failure.* **2** (6), 692-699 (2009).

24 De Ridder, D., Kilgard, M., Engineer, N., & Vanneste, S. Placebo-controlled vagus nerve stimulation paired with tones in a patient with refractory tinnitus: a case report. *Otology & Neurotology.* **36** (4), 575-580 (2015).

25 Shim, H.J. *et al.* Feasibility and safety of transcutaneous vagus nerve stimulation paired with notched music therapy for the treatment of chronic tinnitus. *Journal of Audiology & Otology.* **19** (3), 159-167 (2015).

26 Chesterton, L.S., Foster, N.E., Wright, C.C., Baxter, G.D., & Barlas, P. Effects of TENS frequency, intensity and stimulation site parameter manipulation on pressure pain thresholds in healthy human subjects. *Pain.* **106** (1-2), 73-80 (2003).

27 Badran, B.W. *et al.* Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS). *Brain Stimulation.* **11** (4), 947-948, doi:10.1016/j.brs.2018.06.003 (2018).

28 Ramsay, R.E. *et al.* Vagus nerve stimulation for treatment of partial seizures: 2. Safety, side effects, and tolerability. First International Vagus Nerve Stimulation Study Group. *Epilepsia.* **35** (3), 627-636 (1994).

29 Farrar, J.T., Young, J.P., Jr., LaMoreaux, L., Werth, J.L., & Poole, R.M. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain.* **94** (2), 149-158 (2001).

30 Badran, B.W. *et al.* Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimulation.* doi:https://doi.org/10.1016/j.brs.2018.04.004 (2018).

31 Bauer, S. *et al.* Transcutaneous Vagus Nerve Stimulation (tVNS) for Treatment of Drug-Resistant Epilepsy: A Randomized, Double-Blind Clinical Trial (cMPsE02). *Brain Stimulation.* **9** (3), 356-363, doi:10.1016/j.brs.2015.11.003 (2016).

32 Bikson, M. *et al.* Limited output transcranial electrical stimulation (LOTES-2017): Engineering principles, regulatory statutes, and industry standards for wellness, over-the-counter, or prescription devices with low risk. *Brain Stimulation.* **11** (1), 134-157, doi:10.1016/j.brs.2017.10.012 (2018).

33 Kraus, T. *et al.* BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of Neural Transmission.* **114** (11), 1485-1493 (2007).

34 Kraus, T. *et al.* CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal–a pilot study. *Brain Stimulation.* **6** (5), 798-804 (2013).

35 Fallgatter, A. *et al.* Far field potentials from the brain stem after transcutaneous vagus

nerve stimulation. *Journal of Neural Transmission.* **110** (12), 1437-1443 (2003).
36 Fallgatter, A.J., Ehlis, A.-C., Ringel, T.M., & Herrmann, M.J. Age effect on far field potentials from the brain stem after transcutaneous vagus nerve stimulation. *International Journal of Psychophysiology.* **56** (1), 37-43 (2005).
37 Polak, T. *et al.* Far field potentials from brain stem after transcutaneous vagus nerve stimulation: optimization of stimulation and recording parameters. *Journal of Neural Transmission.* **116** (10), 1237-1242 (2009).
38 Greif, R. *et al.* Transcutaneous electrical stimulation of an auricular acupuncture point decreases anesthetic requirement. *The Journal of the American Society of Anesthesiologists.* **96** (2), 306-312 (2002).
39 Wang, S.-M., Peloquin, C., & Kain, Z.N. The use of auricular acupuncture to reduce preoperative anxiety. *Anesthesia & Analgesia.* **93** (5), 1178-1180 (2001).
40 Badran, B.W. *et al.* Transcutaneous auricular vagus nerve stimulation (taVNS) for improving oromotor function in newborns. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation.* (2018).
41 Borovikova, L.V. *et al.* Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature.* **405** (6785), 458-462 (2000).
42 Ulloa, L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nature Reviews Drug Discovery.* **4** (8), 673-684 (2005).

Figure 1

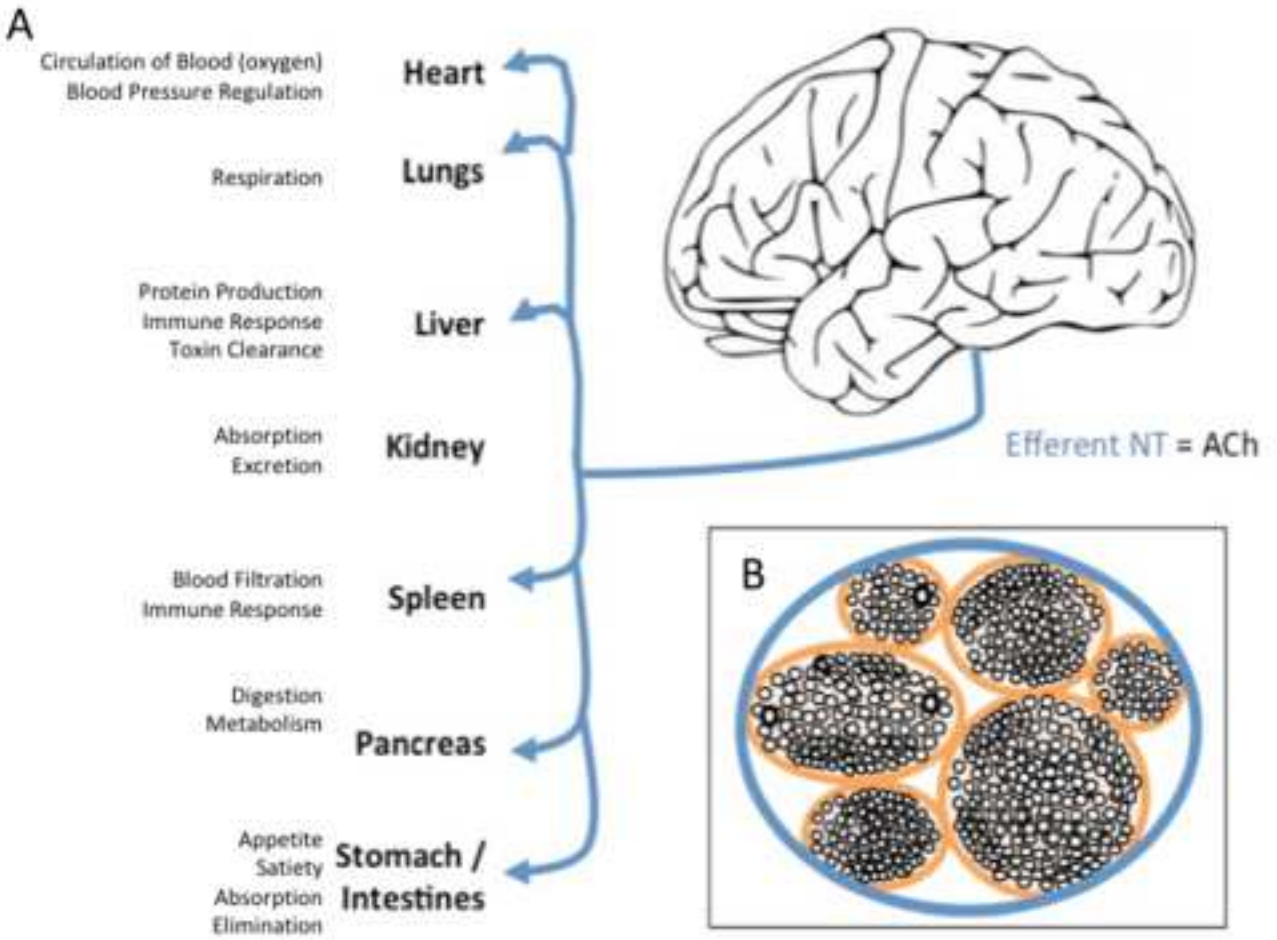


Figure 2

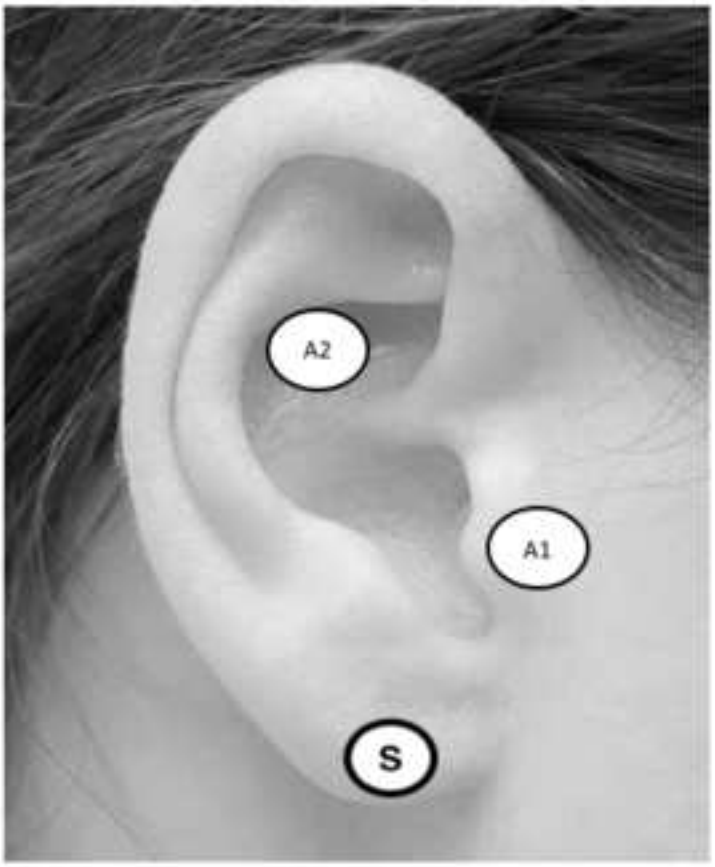


Figure 3

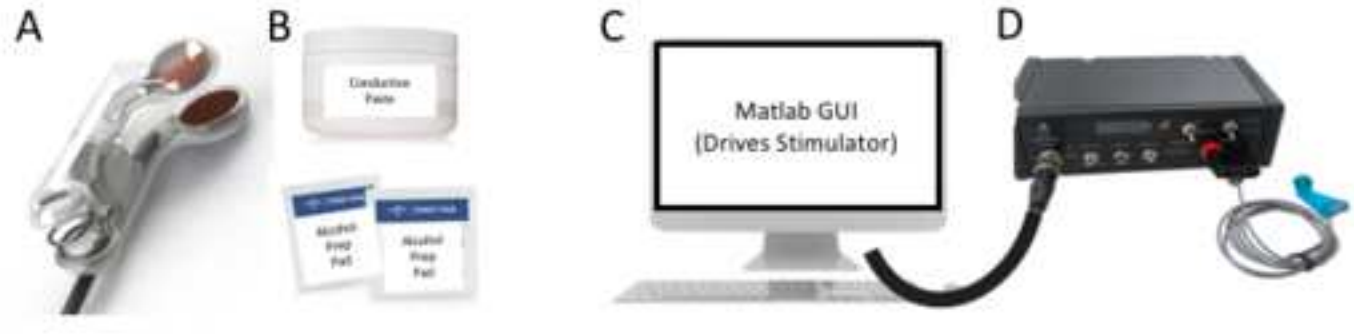


Figure 4

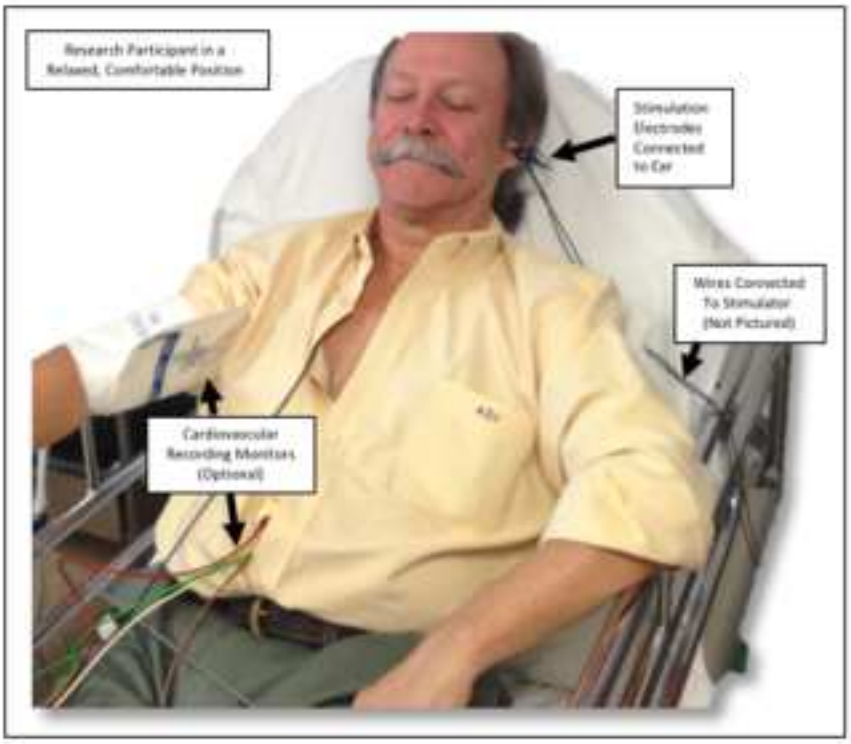


Figure 5

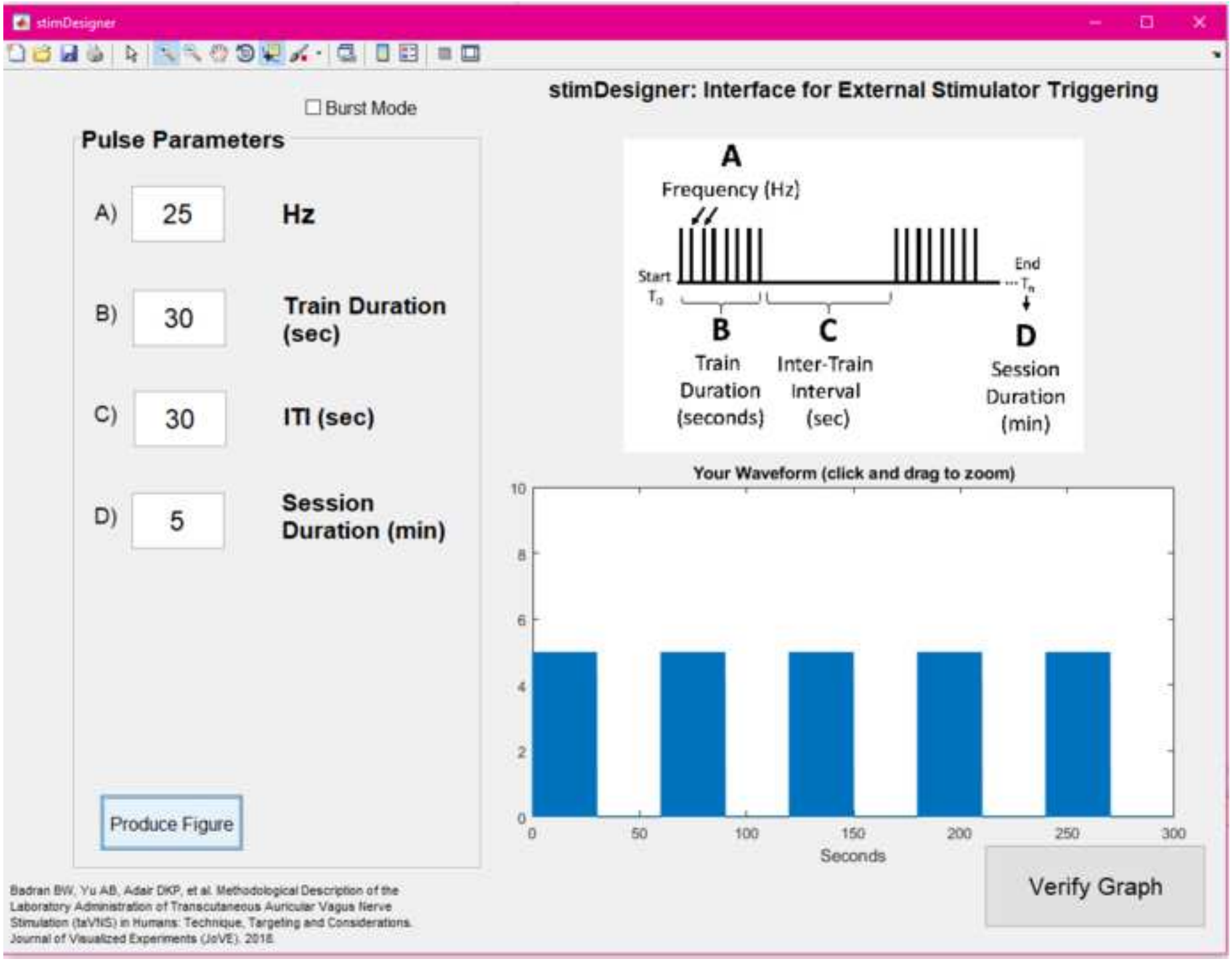
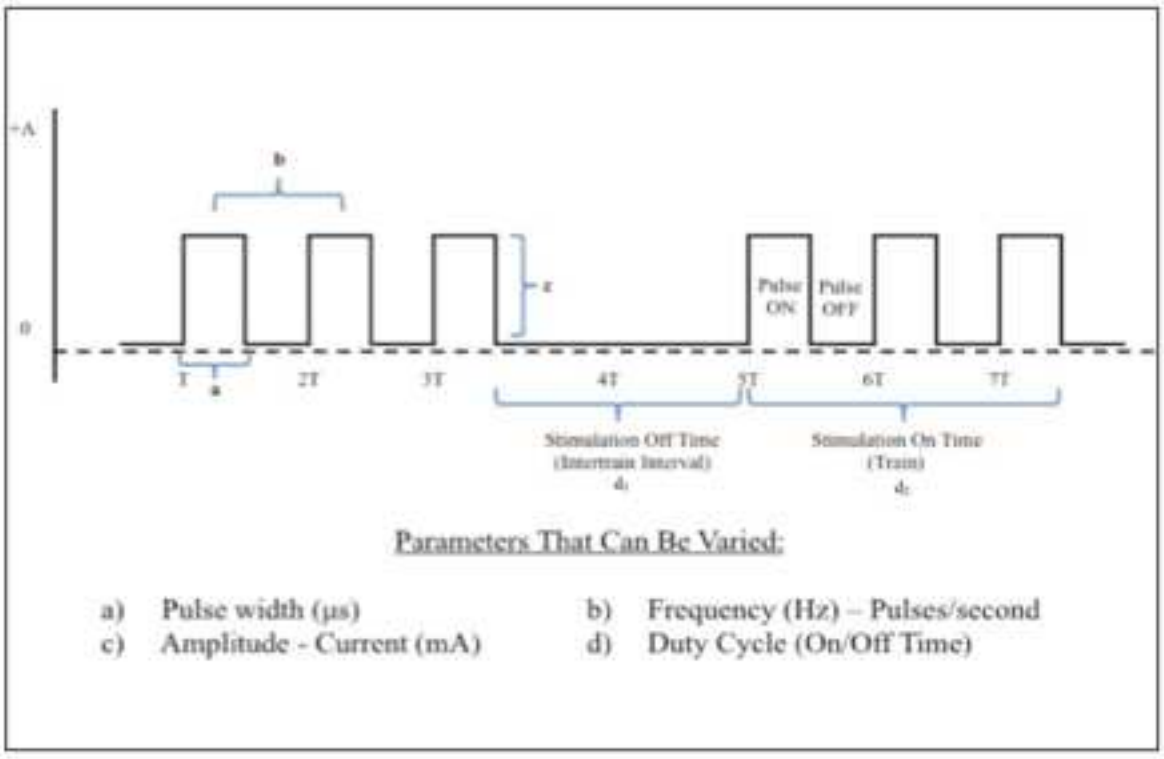


Figure 6

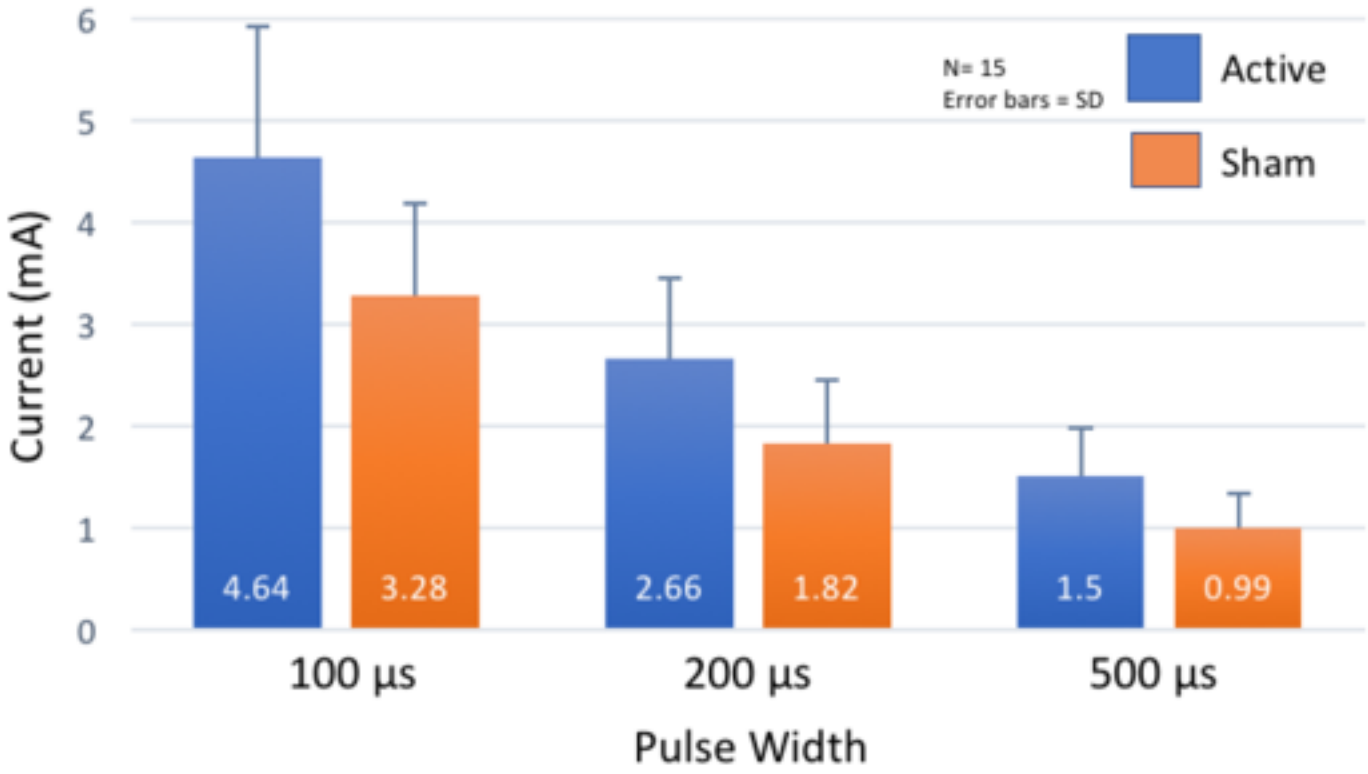


Parameters That Can Be Varied:

a) Pulse width (μs)
b) Frequency (Hz) – Pulses/second
c) Amplitude - Current (mA)
d) Duty Cycle (On/Off Time)

Figure 7

Click here to access/download;Figure;Figure 7.png



Supplemental File

Click here to access/download

Video or Animated Figure

stimDesigner.fig

Supplemental File

Click here to access/download

Video or Animated Figure

stimDesigner.m

Table 1

Stimulation Current Setting	3 mA	1.5 mA	0.75 mA	1.1 mA	0.55 mA	0.825 mA
Response	Yes	Yes	No	Yes	No	Yes

Table 2

	Stim. Current ± SD (mA)		
Pulse Width	**Tragus (Active)**	**Earlobe (Control)**	Sig
100 µs	9.28 ± 2.56	6.57 ± 1.83	Yes
200 µs	5.32 ± 1.60	3.64 ± 1.26	Yes
500 µs	3 ± 0.93	1.97 ± 0.71	Yes

Table 3

	PT ± SD (mA)		Sig	Stimulation Current ± SD (mA)		Sig
	Active	**Control**		**Active**	**Control**	
500 ms 25 HZ	0.82 ± 0.41	0.99 ± 0.39	No	1.37 ± 0.68	1.83 ± 0.73	Yes

Pain ± SD		Sig
Active	**Control**	
1.98 ± 0.83	2.17 ± 1.27	No

Table of Materials

Click here to access/download;Table of Materials;Table of Materials.xlsx

Name of Material/ Equipment	Company	Catalog Number
70% Isopropyl Alcohol Wipes	Any	N/A
Constant Current Stimulator (Triggerable)	Soterix Medical	N/A
Disposable Conductive Electrodes	Custom Built	N/A
Matlab Software w/ Stimulation GUI	MathWorks	N/A
Ten20 Conductive Paste	Weaver and Company	N/A

Comments/Description
Any alcohol preparation pads used for skin in appropriate.
Stimulator manufactured for custom use by Soterix Medical
Stimulation electrodes are custom built at the City College Neural Engineering Lab (Badran/Bikso
MATLAB used for programing pulse pattern
Conductive paste used for administration of stimulation

n)

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:	Methodological Description of the Laboratory Administration of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans: Technique, Targeting and Considerations
Author(s):	*Bashar W. Badran1,2,3, *Alfred B. Yu2, Devin K.P. Adair1, Georgia Mappin3, William H. DeVries3, Dorothea D. Jenkins4, Mark S. George3,5,6, Marom S. Bikson1

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☐ Standard Access ☑ Open Access

Item 2: Please select one of the following items:

☐ The Author is **NOT** a United States government employee.

☑ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee. Alfred Yu

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Bashar W. Badran
Department:	Psychiatry
Institution:	Medical University of South Carolina
Title:	Neuroscientist
Signature:	
Date:	Oct 23, 2018

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

MUSC
DEPARTMENT *of* PSYCHIATRY
& BEHAVIORAL SCIENCES

Brain Stimulation Laboratory

67 President Street
Room 502 North
MSC 861
Charleston, SC 29425
Tel: 843-876-5142
Fax: 843-792-5702

http://academicdepartments.musc.edu/psychiatry/research/bsl/index.htm

Dear Editor,

Attached is our revised mansuscript (revised title: Methodological Description of the Laboratory Administration of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans: Technique, Targeting and Considerations).

We have included a tracked changes and clean version along with responses to all reviewer comments.

Sincerely,

Bashar W. Badran
e. basharwbadran@gmail.com
p. 843-792-1006

Editorial comments:
Changes to be made by the Author(s) regarding the written manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

OK, Completed

2. Please revise the title to reflect the method and its application.

Revised

3. Please provide an email address for each author.

Added to cover page

4. Keywords: Please provide at least 6 keywords or phrases.

Corrected.

5. Please spell out each abbreviation the first time it is used.

Corrected.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Ten20, Matlab, CaviCide, etc.

Corrected. Thank you.

7. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution’s human research ethics committee.

Added.

8. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Corrected.

9. Please revise the protocol to contain only action items that direct the reader to do

something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

Corrected.

10. 1.1-1.4: The Protocol should contain only action items that direct the reader to do something. Please either write the text in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.), or move the materials and equipment information to the Materials Table.

Corrected

11. 2.1: What are the inclusion/exclusion criteria for recruiting the participants?

Added.

12. 2.3, 3.1, 3.5, 4.1, 4.2, 4.5, 5.3-5.6, 6.1, etc.: Please write the text in the imperative tense. Any text that cannot be written in the imperative tense may be added as a “Note.”

Corrected.

13. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below:
3.2: What is used to apply the conductive Ten20 paste?
3.3: How to verify the polarity of electrodes?
5.2: Please specify the pulse generating GUI used in this step.

Added more descriptions to the procedures.

Note- We would like to make the GUI open access. How do we do this? Can you host it as supplemental material?

14. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .pdf, .svg, .eps, .psd, or .ai file.

Corrected.

15. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file.

Corrected.

16. Figure 1: Please use capitalized letters A and B for panel labels.

Corrected.

17. Figure 2: Please note that the sham stimulation site is labeled as S, not B. Please revise to be consistent.

Corrected.

18. Figure 3: Please remove the picture containing commercial language (Ten20 in panel B).

Fixed.

19. Figure 6: Please include a space between numbers and their units (100 µs, 200 µs, 500 µs). Please define SD in the figure legend.

Fixed.

20. Tables 1 and 3: Please include a space between numbers and their units (3 mA, 1.5 mA, 500 µs, etc.). Should 0.825 in Table 2 be followed by mA?

Corrected.

21. Please number the tables in the sequence in which you refer to them in the manuscript text.

Corrected.

22. Please remove the embedded Table of Materials from the manuscript.

Removed.

23. References: Please do not abbreviate journal titles.

Corrected.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
This is a useful protocol that may extend the application of the tVNS, the following are my comments.

Major Concerns:
1. My main concern is the location of the stimulation. Based on a recent manuscript published in brain stimulation (2018) by Andreas M. Burger, there may be no vagus nerve distribution at tragus. Thus, before new evidence come up demonstrating that there is vagus nerve distribution at tragus, it may be not a good idea to put electrode at tragus as shown in Figure 2.

> The reviewer is correct in addressing a current debate as to localization of stimulation. Our group, and others have chosen to stimulate the tragus as an active stimulation, whereas others stimulate the cymba conchae.
>
> This was addressed in the introduction (lines 86-89)
> *The field is still determining optimal stimulation targets[21], although the two most common placements are the anterior wall of the outer ear canal (tragus) and the cymba conchae. Sham stimulation may be conducted by stimulating the earlobe of the ear, an area believed to have minimal ABVN innervation* ***(Figure 2)****.*
>
> We have added a second active site on Figure 2 to clarify this statement.

2. Individuals' s response to electrical stimulation varies significantly. I would suggest the authors do not use the term such as "stimulation current is determined as 200% of an individual perceptual threshold (PT)". There is no evidence suggest that 200% is better than 150% or 250%.

> Great point. We agree. We have changed some of the language in the introduction and softened to language in methods.

3. It seems to the reviewer the threshold selection is too complicated to apply in clinical setting, I would suggest just increasing intensity gradually till the maximal intensity the subject can tolerate, then reduce intensity gradually till subjects feel comfortable.

> The perceptual threshold finding algorithim is similar to the one used in rTMS that was developed and validated over the past 2 decades. This is considered the gold-standard for threshold finding. The authors believe this is an important

dosing parameter and respectfully will be maintaining it as a method for finding PT in the laboratory and clinical trial setting to maintain carefully controlled studies in the infancy of taVNS as a modality.

4. The Figure 4 is confusing, please just include the tVNS related part, delete all other materials that are unrelated to tVNS.

We have changed figure 4 to a more participant-focused image of a laboratory-based taVNS setup,

Reviewer #2:
Introduction
Paragraph 1: There is reference to '(C)' - unclear what this is referring to. Good mention of the financial cost of VNS, would also be worthwhile mentioning the safety profile of VNS, especially as safety and tolerability of taVNS is a key part of the paper.

Great catch. Typo deleted.
Added the safety profile of VNS to the introduction.

Paragraph 2: Mention could be made to the work by Colzato and colleagues- cognitive and social effects of taVNS.

We have added these. Thank you for the suggestion.

Paragraph 3: First sentence - should be 'is' instead of 'in'.

Fixed. Thanks.

Evidence needed for the tragus and cymba concha being the most popular areas of taVNS administration and for the earlobe having minimal ABVN innervation. Further explanation of why the tragus and cymba concha are innervated would be good.

Figure 2 is mentioned for sham stimulation, but not for stimulation on the tragus. Would help to make it clear in this paragraph that Figure 2 also shows where the tragus is. The text also mentions the cymba concha, but this is not illustrated on Figure 2. Another common target is the concha, but this is not mentioned - perhaps worth mentioning?

- R: A literature review reveals these two as the most common treatment positions for taVNS with the earlobe as the most common sham. Intervention is listed in citation 5.

Authors (and the field likely) concede that the field is operating under the auspices that the sole anatomical dissection study – Citation 5. We have made a statement to acknowledge this.

Peuker, E.T. & Filler, T.J. The nerve supply of the human auricle. *Clinical Anatomy.* **15** (1), 35-37 (2002).

We have fixed figure 2 to reflect two active sites and described in the figure legend.

May also be worth mentioning that sham can also consist of attaching electrodes to the same part of the ear which active stimulation is applied, but without passing current despite volunteers believing they are receiving active stimulation.

Interesting. Passive control added to the sham section.

Paragraph 4: Are there any reported side-effects of taVNS? - Left vs. right stimulation. Protocol

Well-detailed and clear. Can see this being useful for others. May be worth mentioning from the outset that this protocol seems to be specific for targeting the tragus. Would need to modify how the ear is targeted if administering taVNS to the concha and cymba concha.

Great point. Clarified in protocol and enhanced figure 2.

One concern is about the kit: does not look very portable or very user-friendly compared to others. Looks to be more used in experimental settings. Perhaps this is worth some consideration in the discussion.

Correct. This is a laboratory, non-portable setting that is used for parametric optimization and flexibility with opensource matlab script.

Electrode preparation and placement section:
Good detail provided regarding anode and cathode placement for the tragus. May be worthwhile being completely clear for anode and cathode placement for sham on the ear lobe.

Great point. Added.

Determination of perceptual threshold section is very good - provides a much more controlled way of determining PT.
May be worthwhile providing more detail about what to do if volunteers find taVNS too painful or uncomfortable.

> Thank you. We have added a brief statement regarding this concern.

Delivering stimulation section:
Figure 5: 'd' could be more clearly labelled on the diagram.

> Labeled d on the ON and OFF time. Duty cycle is hard to describe in a figure like this so we labelled d_1 and d_2 on the On/Off times.

If stimulation is typically administered at 200% of PT, reference is needed (5.3).

> Fixed and noted.

After taVNS section:

Paragraph 1: References needed for first sentence to show that discomfort and side effects of taVNS are 'commonly' reported.

> Added.

Representative results
It is interesting that for the same pulse width, a lower current is needed to sham site (earlobe) than tragus stimulation. From the values presented in Table 2, some of these active vs. sham differences may be statistically significant, would be worthwhile making this clear in this table.

> Added. Thanks for pointing out this oversight.

Would also be of interest to see the pain rating for some of the other stimulation parameters for active and sham stimulation.

> Pain ratings for other parameters can be found in the citation added to the manuscript.

Paragraph 1: There seems to be a slight mix-up with the numbering of the tables. More information about the participants would be helpful (e.g. number of males/females, age range, exclusion criteria).

> Fixed.

Paragraph 2: NRS should be written out in full here (numeric rating scale). References are needed to support the first sentence. Check grammar of third sentence. More information about the participants would be helpful (e.g. number of males/females, age range, exclusion criteria).

Fixed.

Paragraph 3: Sentence 1 needs references.

Added.

Paragraph 4: Check grammar and references are needed.

Fixed typos as a result of merged edits. Thank you.

Discussion
Critical steps in the section:

Bullet point 1: What about mental health conditions? Why screen for cardiovascular disorders? Are there any particular cardiovascular disorders which may prevent participation in a taVNS study?

Inclusion/exclusion criteria included in the protocol. We had accidentally omitted this section in the initial submission.

Bullet point 2: Is there any evidence that their proposed skin preparation improves safety of taVNS? It is already a safe stimulus, so would be interesting to know how this further enhances safety.

Great question. Skin prep reduces resistance at the skin/electrode interface. This allows for less voltage needed to drive the required current through the ear. Generally, we like to use low-current and low-voltage stimulators, to minimize the electricity needed to stimulate the nerve.

Bullet point 3: 'LOTES guidelines' should be expanded and further explained. Also, the group's published guidelines should be referenced.

Lines 47-0481 expand on LOTES and it is cited (#29).

Modifications and troubleshooting of the method section:
What about negative effects encountered by participants?

In our series of studies with over 150 individuals receiving stimulation over 4 studies, we have yet to have a negative effect encountered by participants.

Reviewer #3:
Manuscript Summary:
The manuscript provides a relatively clear description of how to apply a new and promising technique that allows for a non-invasive stimulation of the vagus nerve

Minor Concerns:
1) It may be useful to extend the introduction to mention some of the recent studies that have successfully used taVNS to alter cognitive and social functioning in healthy participants.

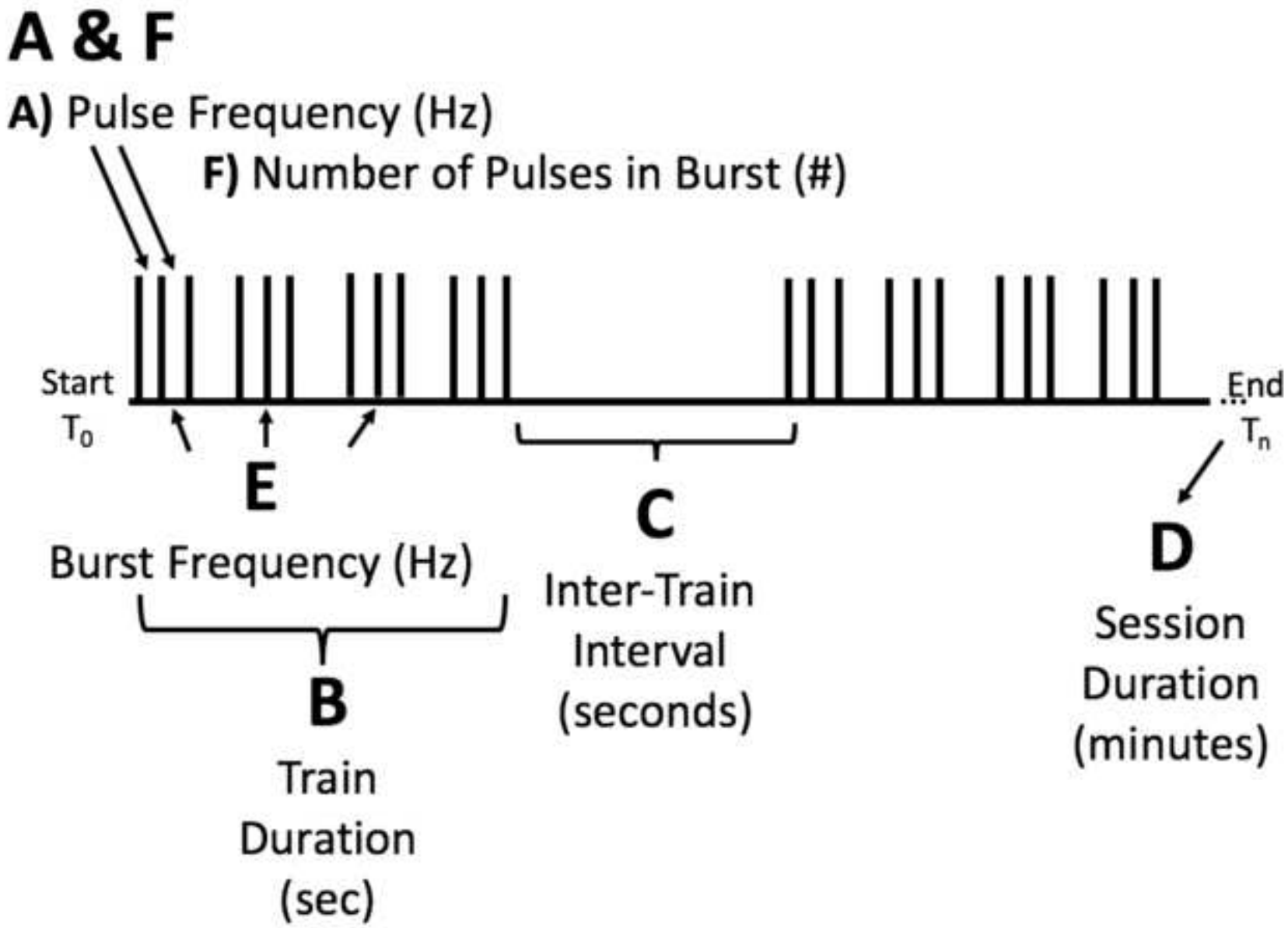
> We have added a brief bit in the introduction about this. Thank you for the suggestion.

2) the main differences between the authors' setup and the stimulation set-ups used by other groups should be pointed out

> Noted. We have discussed different active placements with citation in the introduction and modified figure 2 to reflect other active position some groups use.
>
> We also softened language regarding perceptual thresholds and current intensities as parameters are not yet fully established

3) It would be useful to share the script used to control the stimulator

> We will be including the GUI as an open-source resource with this manuscript. Thank you for the suggestion.



READ ME

Please

Hi Researcher :)

Thank you for using stimDesigner. This software is used to trigger stimulators (electrical, magnetic, etc) by delivering individually designed waveforms custom built by you.

STEP 1: Enter parameters

This software will initiate a series of TTL pulses that drives your stimulator. In order to do this, you must input your desired waveform parameters into the GUI.
These parameters are visualized in the demo waveform figure on the right hand side of the GUI and listed below:

1- Frequency (Hz). This is the number of pulses delivered per second.
2- Train Length - This is the "ON" time of each stimulation train (Input in Seconds)
3- Inter-train interval - This is the "OFF" time between train. (Input in Seconds)
4- Total Duration- This is the length of your desired experimental stimulation period. (Input in MINUTES)

Advanced users may choose to do burst patterns. In that case, please check "burst mode" and the parameters will change to include burst parameters.

STEP 2: Inspect your waveform.

After inputting desired parameters into the GUI, click "PRODUCE FIGURE" to visualize you waveform. This will appear below the demo waveform on the right side of the GUI.

To zoom and inspect your waveform use the magnifier button on the very top tool bar (top left) of the GUI. After clicking this button, use your cursor to drag and inspect the waveform.

STEP 3: Initiate Stimulation

After verifying your waveform is correct in step 2, click "verify" to confirm and subsequently press "ok" in the dialog box. Only after these two steps are complete can you then click "START"

Key Features:

1) There is a running clock that starts as soon as you hit the START button. This counts up to your total session duration.

2) There is a STOP button to stop the triggers. You cannot restart after hitting start, but ensure that you have noted the time you stopped stimulation, exit the GUI, and restart.

Thank you for using stimDesigner. Go forth and stimulate safely. Please cite our manuscript listed below when you use our software. It helps us out!

Badran BW, Yu AB, Adair DKP, et al. Methodological Description of the Laboratory Administration of Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) in Humans: Technique, Targeting and Considerations. Journal of Visualized Experiments (JoVE). 2018.

FAQs and Considerations

In order to properly use stimDesigner, you will need the following software and hardware:

Software:

1- Matlab (tested and built on v2017 but will likely work on other modern versions post-2014)

2- Data Acquisition Toolbox (Matlab toolbox)

3- Update drivers from National Instruments (These drivers are to ensure proper computer/DAQ connectivity)

4- stimDesigner.m & stimDesigner.fig (please keep all files in the root stimDesigner folder)

Hardware

1- PC Computer that can run Matlab (This has not been tested on Linux/Unix/Mac)

2- DAQ (our group uses the NI-DAQmx from national instruments)

3- Stimulator that accepts TTL pulses in for triggering (eg: Digitimer, Grass, Soterix, Magventure, etc)

4- USB cable (PC to DAQ) and a BNC cable (DAQ to Stimulator)

-> Please note this is a research investigational tool for IRB approved investigational studies in the research setting.

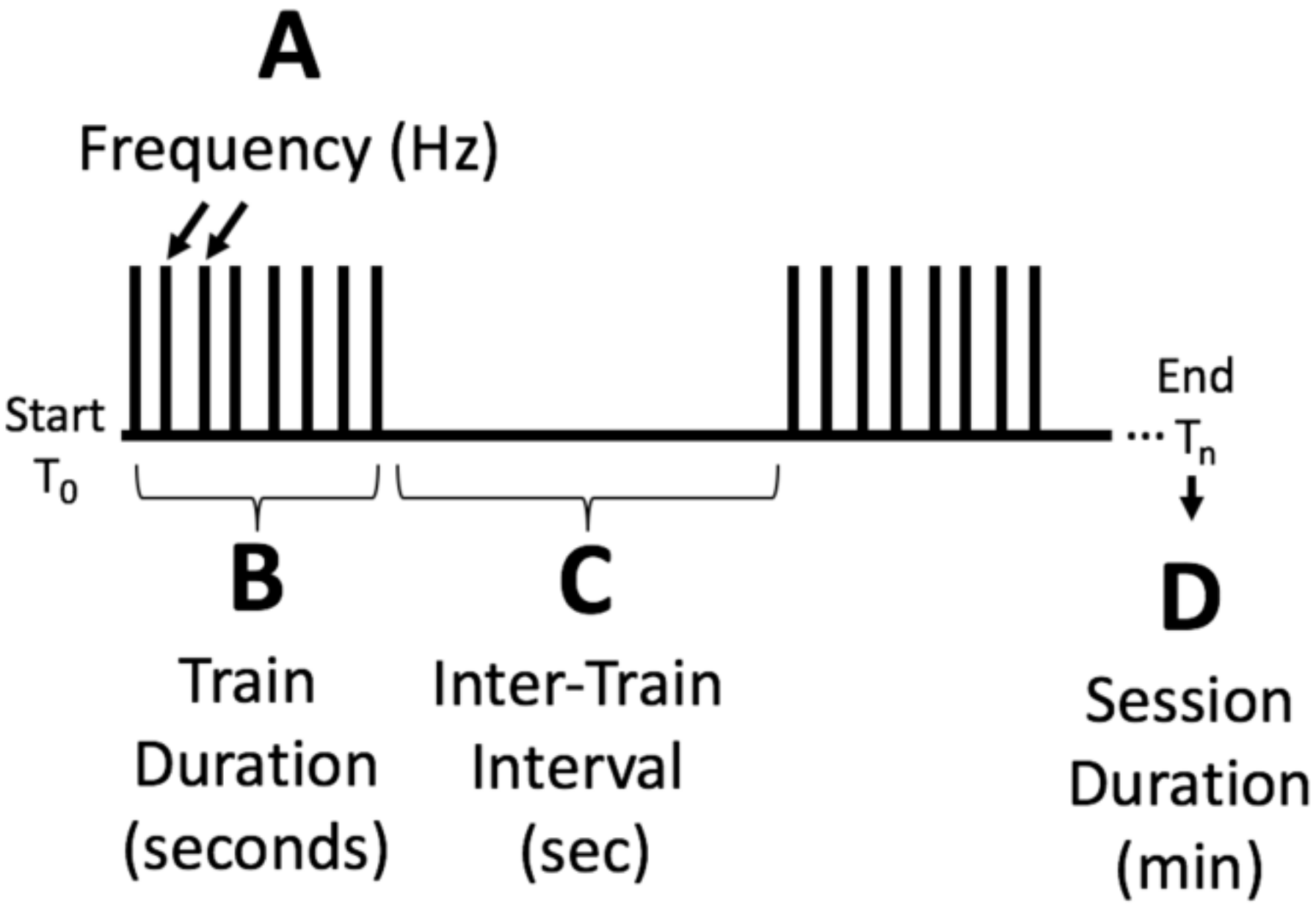
-> Test your output waveform before delivering it to any research participants. Please follow all local guidelines for safe administration of any form of brain stimulation.

Refer to all stimulation safety guidelines to assist in determination of safe stimulation parameters.

Triggering information:
Voltage of the TTL pulse is 5V rising edge (5 microsecond pulse width)

Need Help?

This GUI was designed by Devin Adair of the Neural Engineering Group at CCNY. Feel free to contact her with questions:
dadair@gradcenter.cuny.edu



Supplemental File

Click here to access/download;Supplemental File (Figures, Permissions, etc.);stimDesignerImage.PNG