TRANSCUTANEOUS CERVICAL VAGUS NERVE STIMULATION FOR
OPIOID USE DISORDER

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Presented to
The Academic Faculty

By

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TRANSCUTANEOUS CERVICAL VAGUS NERVE STIMULATION FOR
OPIOID USE DISORDER

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ iii
LIST OF TABLES .................................................................................................................... vii
LIST OF FIGURES .................................................................................................................. viii
LIST OF ABBREVIATIONS ...................................................................................................... x
SUMMARY ............................................................................................................................ xiii
CHAPTER 1: INTRODUCTION ................................................................................................. 1
CHAPTER 2: BACKGROUND ................................................................................................. 3
  2.1 Overview ............................................................................................................................ 3
  2.2 Opioid Use Disorder ......................................................................................................... 3
  2.3 Opioid Withdrawal: Physiological Basis ......................................................................... 4
  2.4 Opioid Withdrawal: Current Treatments ......................................................................... 7
  2.5 Vagus Nerve Stimulation .............................................................................................. 10
  2.6 Vagus Nerve Stimulation for Opioid Withdrawal .......................................................... 12
CHAPTER 3: CLINICAL STUDY DESIGN ............................................................................ 14
  3.1 Noninvasive Biomarkers of Autonomic Nervous System Activity .................................. 14
  3.2: Hardware Selection ....................................................................................................... 17
  3.3 Protocol Design ............................................................................................................... 23
  3.4 Surveys & Subjective Measures .................................................................................... 26
  3.5 Recruitment & Screening ............................................................................................... 27
CHAPTER 4: CLINICAL STUDY RESULTS ......................................................................... 30
  4.1 Subject & Study Information ......................................................................................... 30
  4.2 Data Processing & Analysis ......................................................................................... 33
  4.3 Statistical Analysis .......................................................................................................... 36
  4.4 Results ............................................................................................................................. 37
  4.5 Discussion ....................................................................................................................... 45
CHAPTER 5: CONCLUSION & FUTURE WORK ................................................................ 50
  5.1 Conclusion ...................................................................................................................... 50
  5.2 Limitations ...................................................................................................................... 51
  5.3 Future Work .................................................................................................................... 51
REFERENCES ......................................................................................................................... 54
LIST OF TABLES

Table 1  Anthropometric information per participant. F, female; M, male; BMI, body mass index. 32

Table 2  Anthropometric information and physiological parameter information per device group. P: p-value for the comparison of participants’ characteristics between groups, calculated from student’s t-test for normal continuous variables, Wilcoxon rank-sum test for non-normal continuous variables, or chi-squared test for categorical variables. Normality was assessed using Shapiro-Wilk test. Values represent mean (SD). F, female; HR, resting heart rate; BMI, Body Mass Index. 32
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Withdrawal affects bodily systems including the nervous system, cardiovascular system, respiratory system, and immune system.</td>
<td>7</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Neurophysiological justification of tcVNS for opioid use disorder. tcVNS targets several similar brain structures, including the nucleus tractus solitarus, amygdala, ventral striatum, anterior cingulate, hypothalamus, and locus coeruleus. NTS: nucleus tractus solitarus.</td>
<td>13</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Sensor setup diagram. ECG, electrocardiogram; SCG, seismocardiogram; RSP, respiration belt; IP, impedance pneumography; PPG, Photoplethysmogram; EDA, electrodermal activity.</td>
<td>23</td>
</tr>
<tr>
<td>Figure 4</td>
<td>The protocol consisted of eight different protocol ‘blocks.’ The first two blocks involved neutral videos without stimulation; the second two blocks involved sham or active stimulation without any audiovisual cues; the fifth and seventh blocks were preceded by an opioid cue induction audio; the fifth, sixth, seventh, and eighth blocks consisted of paired stimulation with audiovisual opioid craving cues. Between each protocol block, several measurements were made, including qualitative surveys and blood pressure.</td>
<td>25</td>
</tr>
<tr>
<td>Figure 5</td>
<td>CONSORT diagram displaying enrollment figures. Eight subjects were excluded from participation due to either declining to participate or not meeting inclusion criteria. Of the subjects who participated in the study, two were excluded from analysis; one was due to incomplete data (the subject withdrew from the study partway through data collection) or missing data (hardware malfunction).</td>
<td>31</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Survey results for all subjective survey scores. The top row (NRS-Pain, SUDS, and VAS-Withdrawal) all have significant p values (less than 0.05) while the bottom row (COWS, VAS-Craving, and VAS-Anxiety) all have insignificant p values (greater than 0.05).</td>
<td>38</td>
</tr>
</tbody>
</table>
Figure 7  Δ heart rate, Δ low frequency to high frequency content of the ECG (LFHF ECG) ratio and Δ inverse of the pulse transit time with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. All three biomarkers show significant differences between the active and sham stimulation groups. Heart rate and LFHF ratio were both lower in the active than the sham group; the inverse of pulse transit time was higher in the active than the sham group.

Figure 8  Δ pulse arrival time (PAT), Δ pre-ejection period (PEP), and Δ PPG amplitude with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. None of the biomarkers show significant differences between the active and sham stimulation groups.

Figure 9  Δ pNN50, Δ root mean square of the standard deviation (RMSSD), and Δ respiratory sinus arrhythmia (RSA) with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. None of the biomarkers show significant differences between active and sham groups.

Figure 10  Δ inhalation time and Δ inhalation to exhalation time ratio with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. Both biomarkers show significant differences between active and sham groups; inspiration time and inspiration to expiration time ratio are both lower in the active than sham group.

Figure 11  Δ exhalation time and Δ respiration rate with p value. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. Neither exhalation time nor respiration rate show significant differences between active and sham groups.
LIST OF ABBREVIATIONS

AC Alternating Current
Ag/AgCl Silver/Silver Chloride
ANS Autonomic Nervous System
AU Arbitrary Unit
BMI Body Mass Index
Bpm Beats Per Minute
CONSORT Consolidated Standards of Reporting Trials
COWS Clinical Opiate Withdrawal Scale
DSM-5 Diagnostic And Statistical Manual Of Mental Disorders, Fifth Edition
ECG Electrocardiogram
EDA Electrodermal Activity
EOS Endogenous Opioid System
F Female
FDA Food And Drug Administration
FIR Finite Impulse Response
GABA Gamma-Aminobutyric Acid
HRV Heart Rate Variability
Hz Hertz
IP Impedance Pneumography
kHz Kilohertz
LFHF  Low Frequency To High Frequency Ratio

M  Male

MADs  Median Absolute Deviations

ms  Milliseconds

NRS-Pain  Numerical Rating Scale For Pain

NTS  Nucleus Tractus Solitarus

OUD  Opioid Use Disorder

PAT  Pulse Arrival Time

PEP  Cardiac Pre-Ejection Period

pNN50  Percentage Of Differences in Successive Normal Sinus Intervals That Are Over 50ms

PPG  Photoplethysmogram

PTSD  Post-Traumatic Stress Disorder

PTT  Pulse Transit Time

$PTT^{-1}$  Inverse Of Pulse Transit Time

RMSSD  Root Mean Square Of Standard Deviation

RSA  Respiratory Sinus Arrhythmia

RSP  Respiration Belt

SCG  Seismocardiography

SD  Standard Deviation

SUDS  Subjective Units Of Distress Scale

tcVNS  Transcutaneous Cervical Vagus Nerve Stimulation

tVNS  Transcutaneous Vagus Nerve Stimulation
V Volts

VAS-Anxiety Visual Analog Scale for Anxiety
VAS-Craving Visual Analog Scale for Craving
VAS- Withdrawal Visual Analog Scale for Withdrawal
VNS Vagus Nerve Stimulation
SUMMARY

In the United States, opioid use disorder is quickly becoming a leading cause of death and a public health emergency. Opioid dependence is incredibly debilitating and pervasive; even if patients would like to end opioid use, the extreme withdrawal symptoms often discourage patients. Medication assisted treatment of opioid use disorder is the current gold standard for patient care, but patients must undergo a ‘washout’ period wherein they are unable to use opioids or begin medication assisted treatment and are particularly susceptible to withdrawal symptoms and accidental overdose. Transcutaneous cervical vagus nerve stimulation (tcVNS) is a treatment modality that has been proposed for opioid use disorder patients during this period of early abstinence, as this treatment effects the same brain regions that are responsible for withdrawal and craving symptoms. Additionally, tcVNS offers a device-based (rather than medication-based), noninvasive, low-risk, inexpensive option for treatment of opioid use disorder.

This thesis outlines a double-blind, sham-controlled, randomized clinical study to determine the effectiveness of tcVNS for patients undergoing acute opioid withdrawal. Several sensors were used to record biosignals and extract biomarkers of autonomic nervous system functionality; additionally, subjective surveys were used to determine patient perception of their withdrawal and craving symptoms. Methodologies of biomarker extraction are explored, and the effectiveness of tcVNS for reducing opioid withdrawal symptoms is assessed. Though more investigation is required, preliminary data suggests that tcVNS is effective in reducing withdrawal symptoms, pain, and
distress; additionally, several biomarkers showed significant differences between active and sham groups, suggesting that autonomic nervous system activity is altered during tcVNS in patients undergoing active opioid withdrawal.
CHAPTER 1
INTRODUCTION

Vagus nerve stimulation is a ‘hot topic’ in research right now. At the last few Society for Neuroscience conferences I have attended, there have been several presentations focused solely on the vagus nerve and the various organ systems it innervates. In fact, while working at BioCircuit Technologies I co-authored a grant to explore the effect that the vagus nerve has on gastric stimulators for gastroparesis patients [1-3]. The Inan Research Laboratory has explored a handful of applications of vagus nerve stimulation, including patient populations with traumatic brain injury, post-traumatic stress disorder, and now opioid use disorder [4-13].

Transcutaneous cervical vagus nerve stimulation (tcVNS) offers patients an affordable, non-invasive, minimal risk way to target the vagus nerve. In the past, vagus nerve stimulation was only administered via an implanted device similar to a pacemaker; any kind of implantation comes with the potential for significant surgery-related risks. However, with the development of auricular and cervical transcutaneous vagus nerve stimulation, the benefits of vagus nerve stimulation are now available for much wider patient populations who otherwise could not afford an invasive surgery. However, there is some controversy over how tcVNS works, and whether tcVNS is actually targeting the vagus nerve [14]. That is one of the reasons that studies like this are so important; the scientific community needs to better understand tcVNS, its mechanisms, and physiological effects on specific patient populations.
Opioid use disorder (OUD) patients are an interesting patient population for use of tcVNS. OUD affects many of the brain areas which the vagus nerve innervate or otherwise effect; additionally, opioid withdrawal is characterized by increases in sympathetic tone, which vagus nerve stimulation is known to counter. This study is the first study of neuromodulation in patients actively undergoing opioid withdrawal; it also includes data from several biosignals to better understand the autonomic nervous system’s response to tcVNS.
CHAPTER 2
BACKGROUND

2.1 Overview

Opioid Use Disorders (OUDs) are an epidemic affecting millions of Americans and opioid overdose is quickly becoming a leading cause of death. One of the main reasons OUDs are so difficult to treat is that withdrawal from opioids is often debilitating and painful; furthermore, there are limited treatments for those seeking respite from withdrawal symptoms. Transcutaneous vagus nerve stimulation (tVNS) is a novel treatment for stress-related disorders and targets the brain structures that cause opioid withdrawal and craving symptoms.

2.2 Opioid Use Disorder

2.2.1: Impact: The opioid crisis affects millions of Americans and killed nearly 100,000 Americans in 2020 alone [15]. The crisis is quickly growing, with opioid-related deaths increasing quickly to epidemic proportions. The epidemic can be traced back to pharmaceutical companies marketing opioids as painkillers that are non-addictive, affordable, and without side effects in the late 1990s and early 2000s [16]. Prescriptions of opioids skyrocketed during this time and remain unnaturally elevated today. A study found that in 2015, over 1/3 of all American adults had used prescription opioids within the past year [17]. Due to the severe withdrawal and craving symptoms experienced by those taking prescription opioids, many patients turn to ‘street’ opioids, including heroin, which is even more addictive than pharmaceutical opioids. Currently, the deaths per year attributed to opioids is greater than all other drug overdose deaths combined [18].
2.2.2: Risk Factors: Risk factors for OUDs largely mirror those of other substance use disorders, with the main difference being prescription opioid numbers. In counties with higher levels of prescribed opioids, there are correlated higher levels of opioid misuse and overdose [19]. Other risk factors include socioeconomic status, affiliation with peers who misuse opioids, mood disorders, drug availability, and social stressors. Those who have experienced childhood neglect and/or abuse are at a heightened risk, as are those whose families have a history of drug misuse. Co-occurring mental or physical disorders, such as depression and/or chronic pain disorders, are another large risk factor given the prevalence of ‘self-medicating’ [20].

Opioid use does not guarantee that a patient will develop an OUD; however, the likelihood of developing opioid dependence is higher than most other forms of substance dependence. OUDs are often characterized by a cycle of quitting and relapse which can last an entire lifetime. This cycle increases chances of overdose and injury following a period of abstinence due to the altered tolerance level of the patient [19].

2.3 Opioid Withdrawal: Physiological Basis

The human opioid system is implicated in physiological control of nociception, learning, memory, emotional behavior, and reward circuit regulation [21]. The endogenous opioid system, or EOS, is comprised of opioid receptors and their corresponding endogenous peptide ligands and is found throughout the central and peripheral nervous systems [21]. They are found in higher concentrations in areas of the brain involved in motivation and reward, such as the ventral tegmental area, nucleus accumbens, prefrontal cortex, hypothalamus, and amygdala [21]. Three main types of
opioid receptors have been identified: μ, δ, and κ. The EOS has been implicated in several forms of substance use disorder, not just OUDs [21].

It has been found that exogenous opioid administration activates the brain’s reward system via activation of opioid receptors in the ventral tegmental area and nucleus accumbens [21]. In the ventral tegmental area, dopamine is released when μ- and δ-opioid receptors are activated [21]. In the nucleus accumbens, μ-opioid receptors are activated and downregulate GABA-inhibition [21]. It has been found that the μ-opioid receptors are the most likely to be targeted during addiction; in fact, morphine is a μ-opioid receptor agonist, and the μ-opioid receptor is thereby the reason for the rewarding properties of morphine [21]. Studies have been conducted demonstrating that animals will self-administer both μ- and δ-opioid receptor agonists, but δ-opioid receptor agonists are 100 times less likely than μ-opioid receptor agonists to be self-administered [21].

Repeated exposure to opioids causes the endogenous opioid system to adapt and become less sensitive to opioids. This leads to tolerance and physical dependence on opioids. Engagement of the glutamatergic pathways from the frontal cortex and allocortex cause craving [19]. The reward, stress, and pain systems are all altered during chronic opioid use, and this creates a reactivity to opioid-conditioned cues and increased emotional distress during withdrawal. This all promotes drug-seeking behavior, as individuals are seeking to avoid emotional and physical distress.

Long-term opioid use also affects the locus coeruleus-norepinephrine system, which ties back to autonomic nervous system regulation. Specifically, opioid withdrawal increases the locus-coeruleus – norepinephrine reflex, causing upregulation of
sympathetic nervous system activity [22, 23]. This contributes to withdrawal symptoms, as it is putting the body in ‘fight or flight’ mode once subjects cease using opioids.

It has been hypothesized that there are three main stages of OUD forming a cycle, each of which are associated with specific major brain regions. The first stage of the cycle is the binge/intoxication stage associated with the basal ganglia; it represents dysfunction of habits [19]. The second stage is the withdrawal/negative affect stage associated with the extended amygdala and characterized by negative emotions [19]. The third stage is the preoccupation/anticipation stage associated with the prefrontal cortex and characterized by executive dysfunction [19]. In chronic opioid use, these stages feed into one another and increase symptoms, causing OUDs.
Withdrawal affects bodily systems including the nervous system, cardiovascular system, respiratory system, and immune system.

**2.4 Opioid Withdrawal: Current Treatments**

Withdrawal from opioids is notoriously unpleasant and is characterized by autonomic hyperactivity. The symptoms include nausea, vomiting, anxiety, chills, insomnia, pain, diarrhea, cramping, and lacrimation (see Figure 1) [19]. Medically supervised withdrawal is common, especially when transitioning to opioid antagonists.

There are currently three medications approved by the Food and Drug Administration (FDA) for treatment of OUDS and for preventing relapse, which include buprenorphine, naltrexone, and methadone [24]. All three medications bind to central μ-
opioid receptors, but differ in their effects at the µ-opioid receptor site [24]. Methadone and buprenorphine are both µ-opioid agonists, while naltrexone is a µ-opioid antagonist [24].

2.4.1: Methadone: Methadone has been used to treat OUDs since 1947, making it the medication longest used for OUD treatment [25]. Methadone is a full µ-opioid receptor agonist, meaning that some of the side effects and ‘euphoria’ experienced during opioid use can be experienced using methadone, including respiratory depression which is the root cause of opioid overdose deaths [24, 25]. Several studies support methadone’s effectiveness in reduction of opioid use, infectious disease transmission, and crime. Methadone is taken orally and has a half-life of approximately 24 hours; treatment typically begins with a small dose and slowly works up to a higher maintenance dose [25]. During this ‘ramp-up’ period, the risk of treatment dropout and accidental overdose if titration is too rapid are high [19, 24]. Methadone is still considered the gold standard for OUD treatment [24, 25].

2.4.2: Buprenorphine: Buprenorphine has been much more recently approved for treatment of OUDs in 2002. Buprenorphine is a partial full µ-opioid receptor agonist, meaning it prevents side effects traditionally associated with opioid use such as euphoria or respiratory depression [24]. This means that buprenorphine is safe for rapid induction. Buprenorphine can be either taken alone or in conjunction with naloxone; typically it is given as a sublingual tablet, sublingual film, or buccal film [24]. Given that this is a more recent treatment methodology, there is less literature examining the effectiveness of buprenorphine for OUD treatment; however, the literature that does exist supports its effectiveness [25]. Studies suggest that a longer maintenance dose of buprenorphine is
significantly more effective than buprenorphine for detoxification followed by a placebo [26]. It is also important to note that a sufficiently high dose of buprenorphine must be administered to be effective; some physicians have given lower doses of buprenorphine for shorter than recommended durations, resulting in treatment failure [25].

2.4.3: Naltrexone: The literature on the effectiveness of oral naltrexone is much less convincing than buprenorphine and methadone. In fact, evidence shows naltrexone in daily pill form is not effective in treatment of OUDs, despite its approval by the FDA [25]. Real-world effectiveness of oral naltrexone has been hindered due to poor adherence to the daily medication. Additionally, studies have found significant opioid overdose mortality rates among subjects who discontinued oral naltrexone use [24]. However, a new form of naltrexone administration, extended-release injectable naltrexone, has evidence to support its effectiveness. This form of naltrexone is injected once monthly, reducing the burden on the patient to remember a daily pill. This form of naltrexone was approved by the FDA in 2010. A study which concluded in 2018 found that the extended release naltrexone injection was as effective at reducing relapse as buprenorphine-naloxone [27]. Side effects of the extended-release naltrexone injections include insomnia, reactions at the injection site, hypertension, and influenza [24].

Induction of naltrexone can only occur because opioid agonists must be completely metabolized prior to dosing to avoid severe opioid withdrawal due to its nature as an opioid receptor antagonist. This typically means there is a 7-14 day mandatory abstinence period before beginning treatment with naltrexone; this leads to a significant potential for relapse and treatment dropout, as patients are experiencing opioid withdrawal for days to weeks without medication to alleviate symptoms [24].
2.4.4: Summary: Methadone and buprenorphine are the most commonly used medications for treatment of OUDs and opioid withdrawal [19]. At sufficiently high doses and upon stabilization, both medications attenuate the effects of non-prescribed opioids, reduce craving, and subdue opioid withdrawal symptoms. As opioid receptor agonists, both induce physiological opioid dependence and can lead to withdrawal symptoms if discontinued [24]. Injectable extended-release naltrexone is becoming a more commonly accepted treatment for OUDs, but it cannot help with active withdrawal symptoms due to the mandatory ‘washout’ period between abstinence from opioid use and initiation of naltrexone treatment.

There is an active need to develop non-opioid therapies for treatment of OUDs, including treatment of opioid withdrawal symptoms [24]. Opioid withdrawal includes ‘flu-like’ symptoms as well as affective and cognitive distress which persist up to 30 days in untreated opioid abstinence. This withdrawal often leads to relapse and treatment dropout [24]. Patients experiencing OUDs are often resistant to taking more medication, as medication is often what caused the OUD in the first place.

2.5 Vagus Nerve Stimulation

The autonomic nervous system (ANS) controls and regulates organs, glands, and involuntary muscles. The ANS is comprised of two different components: the sympathetic nervous system, which is responsible for the ‘fight or flight’ response, and the parasympathetic nervous system, which is responsible for the ‘rest and digest’ response [28]. The vagus nerve is the main conduit of the parasympathetic nervous system, and innervates several structures in the body including the heart, lungs, and
gastrointestinal tract [28]. The vagus nerve is comprised of approximately 20% efferent fibers and 80% afferent sensory fibers [28]. The afferent fibers terminate in the nucleus tractus solitarius, which has projections to brain regions including the locus ceruleus, amygdala, hypothalamus, parabrachial nucleus, and dorsal raphe nuclei [28, 29]. Many of these structures are associated with emotional regulation and mood [29].

In the 1880s, an observation was made that a massage of the carotid artery could suppress seizures; this is now attributed to manual stimulation of the vagus nerve [28]. Early electrical vagus nerve stimulation (VNS) studies mainly focused on the feasibility of VNS as a treatment for epilepsy; the first VNS device to be FDA-approved was an implanted VNS device for the treatment of refractory epilepsy in 1997. In 2005, the same device was approved for treatment of chronic treatment-resistant depression [28]. Most implantable VNS stimulation has been performed on the left cervical vagus, as there has been evidence to show that right vagus stimulation may interfere with cardiovascular function [30]. In fact, right cervical VNS has been developed for the treatment of heart failure, but is not yet FDA approved [31]. The implanted stimulators appear similar to a pacemaker, with implantation of a pulse generator which is connected via a lead wire to a cuff electrode which surrounds the vagus.

The need for an invasive surgery and generalized anesthesia for implantable VNS treatment means that it is less accessible to patient populations and has intrinsically more surgery-related side effects [32]. This method of treatment is often used as a ‘last resort’ for patients with no other options [32]. Due to these barriers, noninvasive forms of VNS have been developed to target parts of the vagus nerve that lie near the skin surface. There are two main forms of transcutaneous VNS: auricular and cervical. The outer ear,
specifically the cymba conchae and cavum conchae, is innervated by the auricular branch of the vagus nerve. Stimulators which target this receptive field have been shown to create a brain pattern similar to that of implanted cervical VNS [33]. In Europe, a transcutaneous auricular VNS device has been approved for use in treatment of epilepsy, depression, and pain. Transcutaneous cervical VNS (tcVNS) targets the vagus as it runs along the carotid artery in the neck. A tcVNS device has European regulatory approval for treatment of migraine, cluster headache, and hemicrania continua [28].

2.6 Vagus Nerve Stimulation for Opioid Withdrawal

The vagus nerve has been suggested as a target for OUDs and opioid withdrawal for several reasons. The brain regions that the vagus nerve innervates and that lead to opioid dependence and withdrawal symptoms have significant overlap, as seen in Figure 2. Specifically, the hypothalamus and Locus Coeruleus are responsible for withdrawal symptoms, while the Anterior Cingulate, Ventral Striatum, and Amygdala are responsible for craving and relapse. tcVNS effects all of these areas by way of the Nucleus Tractus Solitarius. The effect of tcVNS on withdrawal symptoms can be measured via noninvasive physiological monitoring to determine the sympathetic nervous system response.
Figure 2: Neurophysiological justification of tcVNS for opioid use disorder. tcVNS targets several similar brain structures, including the nucleus tractus solitarius, amygdala, ventral striatum, anterior cingulate, hypothalamus, and locus coeruleus. NTS: nucleus tractus solitarius.
CHAPTER 3
CLINICAL STUDY DESIGN

In order to investigate the efficacy of tcVNS for OUDs and opioid withdrawal, a robust randomized double-blind clinical trial was designed and conducted. The study had several objectives, including assurance that the protocol was truly double-blind and placebo controlled to ensure data fidelity. The protocol was designed to compare active and sham stimulation during exposure to stimuli known to incur craving and withdrawal symptoms. The sensors were chosen for their ability to provide insight into the autonomic nervous system state during experimentation.

3.1 Noninvasive Biomarkers of Autonomic Nervous System Activity

It is important to understand how the autonomic nervous system reacts to tcVNS; however, the autonomic nervous system is integral in so many organ systems that it is hard to measure in an objective and holistic fashion. Therefore, investigators used specific biomarkers that correlate with autonomic activity to determine various dimensions of autonomic response to a stimulus. Specifically, the investigators were interested in sympathetic versus parasympathetic nervous system activation.

3.1.1 Heart Rate: Cardiovascular activity is highly related to autonomic nervous system activity; heart rate is one of the most widely used measures of cardiovascular activity. In fact, one of the most common symptoms of panic attacks and stressful events is a ‘racing heartbeat.’ Heart rate is quantified as the number of beats per minute (bpm). The heart is innervated by both the sympathetic and parasympathetic nervous systems; a
higher heart rate is correlated with higher sympathetic nervous system activity and/or lower parasympathetic nervous system activity [34].

3.1.2 Heart Rate Variability (HRV): HRV provides another measure of cardiovascular activity. While heart rate measures how quickly the heart beats, HRV measures the variation in heart rate. HRV analysis is commonly completed in the frequency domain; sympathetic activity is associated with a lower frequency range (0.04-0.15 Hz) while parasympathetic activity is associated with a higher frequency range (0.15-0.4 Hz) [35].

3.1.3 Cardiac Pre-Ejection Period (PEP): PEP is a cardiovascular measure of heart contractility and sympathetic activity, defined as the latency between ventricular depolarization and aortic valve opening. Though a PEP value alone does not provide much information on cardiac state, changes in PEP over time can suggest changes in contractility. An increase in contractility leads to an increase in the rate of left ventricular pressure increase during isovolumetric contraction, and therefore a decrease in PEP [36]. Therefore, PEP is inversely related to sympathetic activity.

3.1.4 Pulse Arrival Time (PAT): PAT is a measure of the time between heart depolarization and arrival of the pulse waveform at a peripheral site, often the finger [36]. This requires a multimodal approach to cardiovascular measurement. PAT can provide a correlate of blood pressure, though some other factors (heart contractility) are included in this metric.

3.1.5 Pulse Transit Time (PTT): PTT is defined as the delay of a pulse waveform travelling from one arterial site to another. This biomarker is an indirect measurement of arterial stiffness, and so provides insight into sympathetic control of peripheral
vasoconstriction and blood pressure [12]. In fact, the reciprocal of PTT can be used as a surrogate for blood pressure measurements in wearable systems, and is generally more accurate than PAT [12]. PTT is calculated by subtracting PEP from PAT. An increase in 1/PTT is associated with sympathetic arousal [12].

3.1.6 Blood Pressure: Blood pressure is highly controlled by the autonomic nervous system. Sympathetic arousal increases blood pressure, while parasympathetic activity decreases blood pressure. It is typically measured via an arm cuff, which compresses the arteries in the arm and measures systolic pressure (the pressure placed on the arteries during heart contraction) and diastolic pressure (the pressure placed on the arteries during heart relaxation). High blood pressure is a major cause of cardiovascular failure, and has been linked to hyperactivity of the sympathetic nervous system [37].

3.1.7 Electrodermal Activity (EDA): EDA is a measurement of the electrical conductivity of the skin, which changes with sweat gland activity. Sweat is extremely conductive, and so a higher level of conductance is associated with a higher level of sweat gland activity [38]. Sweat glands, especially those in the hands and feet, are reactive to psychological stimuli as well as thermoregulatory stimulation [38]. Sympathetic arousal leads to an increase in sweat gland activity and therefore an increase in electrodermal activity [38]. EDA is typically separated into tonic (low frequency) and phasic (high frequency) responses [38].

3.1.8 Respiration Rate: Respiratory control is closely associated with the cardiovascular system and sympathetic nervous system [39]. However, there are other feedback mechanisms involved in involuntary respiration control including chemoreceptors and proprioceptors [40]. Increase in sympathetic activity leads to an
increase in respiration rate. It is also interesting to note that since respiration can be voluntarily or involuntarily controlled, studies have shown that controlling respiration rate can increase or decrease sympathetic activity. In fact, breathing exercises are common treatments for psychiatric disorders associated with increased sympathetic activity such as anxiety or panic disorders.

3.1.9 Inhalation and Exhalation Time and Ratios: Respiration metrics more nuanced than respiration rate are slowly becoming more recognized as important markers of sympathetic tone [13, 39]. During exhalation vagal activity (and therefore parasympathetic activity relative to sympathetic activity) increases, while vagal activity decreases during inhalation, corresponding to increased sympathetic activity (relative to parasympathetic activity) [13]. Due to the relative sympathetic to parasympathetic activity change during respiration, heart rate increases during inhalation and decreases during exhalation. This phenomenon is known as respiratory sinus arrhythmia and enables respiration metrics to be extracted from heart rate information. As there is differing parasympathetic and sympathetic activity during the phases of respiration, metrics such as inhalation time, exhalation time, and the ratio of inhalation to exhalation time are becoming more recognized as markers of autonomic activity [39].

3.2: Hardware Selection

3.2.1 Stimulator & Sham Control: Both active and sham stimulation devices (gammaCore, electroCore, Basking Ridge, New Jersey) were identical in form and operator interaction; the only way they differed was in waveform applied. The active stimulator waveform consisted of an alternating current (AC) voltage signal of 1ms of a
5kHz sine wave repeated every 40 ms. This means that five periods of the 5kHz sinusoid is applied followed by 39 ms of no signal. The sham device produced a 0.2Hz AC biphasic square wave. The voltage on the active device ranged from 0 to 26V, while the voltage on the sham device ranged from 0 to 4V. Voltage was adjustable via a thumbwheel switch; intensity was indicated on the switch as labels from 0 to 5.

The stimulation device was connected to bar electrodes which were placed over the subject’s right carotid artery. A small amount of electrode gel was applied to the surface of the bar electrodes before placement on the neck; the bar electrodes were held in place by a small fabric strap placed around the neck.

At stimulation onset, research personnel turned the device on via the thumbwheel switch; visual and audio confirmation of operation was provided by an LED and an audible beep. The researcher would then ramp up stimulation intensity using the thumbwheel while asking the participant to indicate any discomfort. Stimulation intensity was set to the maximum voltage the subject could tolerate without pain. The stimulation device continued applying stimulation for 120 seconds, after which another audible beep was heard and the device automatically ended stimulation.

3.2.2 Data Acquisition System: The Biopac Systems MP150 data acquisition system (Goleta, CA) was used to obtain, record, and sync all biosignals with the exception of EDA and impedance pneumography. All data collected via the MP150 was acquired at a rate of 2kHz.

3.2.3 Respiration Belt: Respiration belts are a widely used noninvasive form of respiration measurement [41]. Though the gold standard of respiration measurement is a spirometer, it is not always feasible to have subjects breathe into a tube during
experimentation; therefore, the respiration belt is commonly used during experiments where subjects are active or otherwise unable to use a spirometer [41]. A respiration belt consists of a belt of fabric placed around the chest of the subject. There is a strain gauge connected to each side of the belt that senses changes in thoracic circumference as the subject breathes in and out. There are several biosignals that can be derived from respiration belt signals, including respiration rate, inspiration/expiration times, and relative respiration intensity [41]. Since this is a mechanical measurement, movement and subject position are potential corrupting factors to consider. For this experiment, a piezoresistive respiration belt (Biopac PN BN-RESP-XDCR) was used for signal transduction via a dual wireless transmitter (Biopac PN BN-RSPEC-T), and was amplified and collected via RSPEC-R amplifiers from Biopac Systems.

3.2.4 Impedance Pneumography (IP): IP measures the change in impedance of the thoracic cavity as an individual breathes. This method noninvasively measures the electrical conductivity of the lungs by applying a small alternating current at a specific frequency using electrodes placed on either side of the thoracic cavity and measuring the voltage drop using proximal electrodes [42]. Since air is less conductive than the body, as a subject inhales the measured impedance increases. Since this is an electrical measurement, it is less sensitive to physical movement than other forms of respiration measurement including respiration belts. Several respiratory biomarkers can be extracted from IP waveforms, including respiration rate, inspiration time, expiration time, and tidal volume. This study used an IP device designed, assembled, calibrated, and tested by another member of the Inan Research Laboratory, Samer Mabrouk, who has published several papers on his design and calibration techniques [42-44]. Disposable Ag/AgCl
electrodes were used in conjunction with the IP device. The device saved the IP waveform to a memory card, therefore the data was not directly synced with the data collected via the Biopac MP150 system. To adjust, we included a calibration step in the setup of the device: a data collection personnel would physically tap the IP electrodes, which created an artifact in the IP, electrocardiogram, and seismocardiogram signals. This was used in post-processing to sync the IP device with the Biopac data acquisition system.

3.2.5 Electrodermal activity (EDA): EDA information was gathered via a wristwatch-based device (E4, Empatica Inc., Milan, Italy). This device was chosen as it is highly cited for research use [45]. The device also has a built-in reflectance-based photoplethysmogram (PPG) sensor, which can be used to determine heart rate and heart rate variability. The data gathered during the experiments were stored on the Empatica device and later uploaded to the Empatica data storage system; the EDA data was synced with the data gathered via the Biopac system using wall-clock time.

3.2.6 Electrocardiogram (ECG): Electrocardiography is one of the best-studied and widely used forms of measuring cardiac activity. ECG measures the electrical activity of the heart using electrodes placed on the torso. In this experiment, investigators used the Lead I configuration, which utilizes three electrodes as depicted in Figure 3. The positive electrode is placed on the upper left chest, the negative electrode is placed on the upper right chest, and the reference electrode is placed on the lower left abdomen. This configuration provides a ‘classic’ ECG signal with an easily identifiable PQRST complex per heartbeat. ECG is the gold standard for determining heart rate (and therefore HRV), and changes to the PQRST complex can provide information about changing heart...
dynamics [46]. For this experiment, a 3-lead set (Biopac PN BN-45-LEAD3) and disposable Ag/AgCl electrodes were used for signal transduction via a dual wireless transmitter (Biopac PN BN-RSPEC-T); the signal was amplified and collected via an RSPEC-R amplifier and MP150 data acquisition system from Biopac Systems.

3.2.7 Photoplethysmogram (PPG): PPG utilizes optical measurement techniques to determine changes in vascular tissue beds [47]. A PPG sensor consists of a light source to illuminate tissue and photodetector to sense changes in light intensity which indicate changes in blood volume within the microvascular tissue bed. This is most often applied noninvasively, often to a finger or earlobe. The light source is typically at a red or near infrared wavelength; green light sources are occasionally used if the investigators are interested in calculating oxygen absorption. PPG can be recorded via two main methods: transmission and reflectance. Transmission PPG consists of a light source and detector which are separated by tissue, whereas reflectance PPG involves a light source and detector on the same tissue surface to measure reflected light [48]. PPG signals consist of two components: the ‘AC’ component which corresponds to the heartbeat, and the ‘DC’ component which varies much more slowly and corresponds to respiration, vasomotor activity, and vasoconstriction. Several biosignals can be obtained from the PPG waveform, including heart rate, correlates of blood pressure, pulse arrival time when utilized in conjunction with ECG, and pulse transit time when utilized in conjunction with seismocardiography and ECG. For this clinical trial, a transmission-based PPG transducer was used (Nonin Medical Inc., Plymouth, MN; PN 8000AA-3) and a PPG100C amplifier from Biopac Systems was used for signal amplification. An adapter was used to connect the transducer to the amplifier (Biopac PN TCIPPG3).
3.2.8 Seismocardiography (SCG): SCG measures the mechanical activities of the heart, typically using a highly sensitive low noise accelerometer placed on the chest. As such, SCG signals are susceptible to movement-based noise. Typically, dorsoventral acceleration is measured, though all three axes of chest acceleration are studied and relevant to cardiovascular activity quantification. The SCG signal provides a wealth of information about heart activity. Cardiac events including mitral valve closure, isovolumetric contraction, aortic valve opening, rapid ejection, aortic valve closure, and mitral valve opening can be extracted from the SCG waveform; when paired with the ECG, important biomarkers including pre-ejection period can be inferred [49]. For this experiment, a low-noise triaxial accelerometer (PCB Piezoelectronics, 356A32/NC) was connected to a signal conditioner (PCB Piezoelectronics 482C15) via a 4-conductor cable (PCB Piezoelectronics 034K10). The signal conditioner was then connected to the Biopac data acquisition system via an analog input module (Biopac, UIM100C).
Figure 3: Sensor setup diagram. ECG, electrocardiogram; SCG, seismocardiogram; RSP, respiration belt; IP, impedance pneumography; PPG, Photoplethysmogram; EDA, electrodemal activity.

3.3 Protocol Design

3.3.1 Randomization & Blinding: A research personnel who was not involved in data collection, recruitment, enrollment, or data analysis assigned subjects to either ‘active’ or ‘sham’ groups. The assignment was completed via simple randomization, and
the research personnel provided the corresponding stimulation devices to the data
collection team. The stimulation devices were only identifiable by a serial number on the
device; otherwise, the active and sham operated identically. The serial number key was
only revealed to data collection and data analysis personnel upon active vs sham analysis
of the data. As such, data collection personnel and subjects were blinded to active vs
sham groups throughout screening and data collection.

3.3.2 Audiovisual Cues: The opioid cue videos were chosen due to their ability to
induce opioid craving and withdrawal-like symptoms [50]. The videos include imagery
of individuals handling and taking opioids, including pills and injectable substances. The
opioid induction audio consisted of a four-minute guided meditation, including
instructing participants to picture the last time they used and the surroundings at that
point. The purpose of the audio is to induce opioid craving and withdrawal symptoms in
preparation for the stimulation window. The neutral videos involved a woman discussing
her job at the United States Postal Office. The video was chosen for its general neutrality
and lack of reference to opioids. Each video (both neutral and opioid cue) was
segmented into approximately 2-minute sections and shown to the subject as separate
clips.

3.3.3 Protocol Outline: The investigators desired a ‘baseline’ period without
stimulation to be compared to an ‘active’ period with stimulation. Opioid cue videos
were used, due to their ability to increase craving and withdrawal symptoms;
correspondingly, the baseline period included neutral videos which were not meant to
increase symptoms but would recreate the physiological response to exposure to an
audiovisual stimulus. Other audiovisual cues were used to induce craving-like responses.
The protocol includes ‘neutral videos’ which should not elicit a craving response from subjects, stimulation without exposure to craving videos, stimulation with exposure to craving videos, and a craving induction audio prior to some of the stimulation segments. There are eight protocol ‘blocks,’ with at least 5 minutes of time between blocks to allow subjects to return to baseline. After each block, blood pressure is measured and subjective surveys are administered. The first two blocks are neutral videos without stimulation. The third and fourth block are tcVNS stimulation without exposure to videos of any kind. The fifth and seventh blocks are preceded by induction audio and consist of stimulation in conjunction with opioid cue videos. The sixth and eighth blocks are stimulation in conjunction with the opioid cue videos. All stimulation was applied for 2 minutes at a time. Stimulation intensity was determined by subject tolerance; subjects were asked to indicate when the stimulation was uncomfortable and the intensity was set just under the ‘tolerability’ threshold.

Figure 4: The protocol consisted of eight different protocol ‘blocks.’ The first two blocks involved neutral videos without stimulation; the second two blocks involved sham or active stimulation without any audiovisual cues; the fifth and seventh blocks were preceded by an opioid cue induction audio; the fifth, sixth, seventh, and eighth blocks consisted of paired stimulation with audiovisual opioid craving cues. Between each protocol block, several measurements were made, including qualitative surveys and blood pressure.
3.4 Surveys & Subjective Measures

Several subjective measures were taken throughout the study protocol to explore various factors that contribute to opioid dependence and lead to relapse and often overdose during the cessation period. These include withdrawal symptoms, craving, anxiety, and distress. Though there is overlap in some of the scales selected, each scale measures a unique aspect of opioid withdrawal and risk factors.

3.4.1 Clinical Opiate Withdrawal Scale (COWS): The COWS is a scale that considers 11 different withdrawal symptoms including pulse rate, sweating, restlessness, pupil size, bone or joint aches, runny nose or tearing, gastrointestinal upset, tremor, yawning, anxiety or irritability, and piloerection [51]. The COWS is often used clinically to determine withdrawal severity; to determine a COWS score, the various items are scored and added together. The COWS score ranges from 0, for no withdrawal symptoms, to 48, or maximum withdrawal symptoms. A COWS score of 5-12 corresponds to mild withdrawal; 13-24 corresponds to moderate withdrawal; 25-36 to moderately severe withdrawal; and more than 36 corresponds to severe withdrawal [51]. This scale was applied at the beginning and end of the protocol, and again at the 6-week follow-up call.

3.4.2 Numerical Rating Scale for Pain (NRS Pain): Subjects were asked to rate their current pain on a 0-10 scale, with 0 being ‘no pain,’ 5 being ‘moderate pain,’ and 10 being ‘worst possible pain.’ This scale was applied at the beginning and end of the protocol, and again at the 6 week follow-up call. This scale is often used clinically to determine pain levels, and is indicative of specific opioid withdrawal symptoms [52].
3.4.3 Subjective Units of Distress Scale (SUDS): Subjects were asked to rate their current level of distress on a 0-100 scale, with 0 being ‘not at all distressed’ and 100 being ‘most ever.’ This scale was applied at the beginning of the protocol and after every protocol block, for a total of nine datapoints.

3.4.4: Visual Analog Scale for Withdrawal (VAS-Withdrawal): Subjects were asked to rate their current opiate withdrawal symptoms on a 0-10 scale, with 0 being ‘none’ and 10 being ‘severe.’ This scale was applied at the beginning of the protocol and after every protocol block.

3.4.5 Visual Analog Scale for Anxiety (VAS-Anxiety): Subjects were asked to rate their anxiety on a 0-10 scale, with 0 being ‘not at all anxious’ and 10 being ‘the most anxious I’ve ever felt.’ This scale was applied at the beginning of the protocol and after every protocol block. Though similar to the SUDS score, the subjects were asked specifically about their anxiety as opposed to their distress.

3.4.6 Visual Analog Scale for Craving (VAS-Craving): Subjects were asked to rate their current opiate cravings on a scale from 0-10, with 0 being ‘no craving’ and 10 being ‘extreme craving.’ This scale was applied at the beginning of the protocol and after every protocol block.

3.5 Recruitment & Screening

3.5.1 Recruitment: Study recruitment is notoriously difficult among those who are actively undergoing opioid withdrawal. There are several factors for this, including a general distrust of the medical community, taboos associated with illicit drug use, and
socioeconomic factors that may affect the viability of study participation. Additionally, those experiencing OUDs are a very high-risk population, as opioid overdose deaths are all too common. Acute opioid withdrawal is a relatively small window to study – subjects were anywhere from 8 to 24 hours abstinent from opioids. Subjects often did not know more than a day ahead of time that they would be seeking treatment, so recruiting subjects at the point of care was crucial.

As a result of recruitment difficulties, the study team pursued several paths for recruiting subjects. The first subject participant pathway was via Emory Healthcare Addiction Services. Several subjects were referred to us by the Emory team. The second pathway for subject recruitment was via Alliance Recovery Center in Decatur, GA. Alliance was profoundly helpful in referring subjects to the study. As a recovery center which doses buprenorphine and methadone, Alliance sees many patients who are coming in to begin OUD treatment and are in active withdrawal. As such, Alliance clinical staff recommended the study to their patients and connected us with patient contact information if there was interest.

3.5.2 Screening: Subjects were thoroughly screened prior to study initiation to ensure subject safety and to remove potential confounding factors. Individuals who were pregnant or breastfeeding at the time of the study, those who had implanted electronic devices, and those who had a medical history of vagotomy, meningitis, traumatic brain injury, neurological disorder, mental disorder, loss of consciousness greater than one minute, schizophrenia, schizoaffective disorder, bulimia, serious medical or neurological illness, or carotid atherosclerosis were all excluded from study participation. Subjects
were required to be between 18 and 80 years of age and must have met DSM-5 criteria for Opioid Use Disorder at the time of the study.
CHAPTER 4

CLINICAL STUDY RESULTS

4.1 Subject & Study Information

4.1.1 Study Information: The clinical trial (ClinicalTrials.gov NCT04556552) was approved by the institutional review boards of the Georgia Institute of Technology (H20203) and Emory University (IRB00117320). Subjects were screened as described in 3.5.2. All participants provided written informed consent after being provided a full description of the protocol and being given the opportunity to ask questions about the study. Data were collected at either the Alliance Recovery Center or Emory University School of Medicine between November 2020 and September 2021. During data collection, study participants were undergoing acute opioid withdrawal, defined as abstaining from opioids for at least eight hours. Participants were recruited for the study prior to medication assisted treatment.

4.1.2 Subject Information: As shown in the CONSORT diagram in Figure 5, 31 subjects were assessed for study eligibility. Of those participants, 8 were excluded from the study; 5 declined study participation and 3 did not meet study criteria. 23 subjects agreed to participate and met all study inclusion criteria. The subjects were randomized into active and sham groups as described in 3.3.1. One subject did not complete the entire study (withdrew partway through the protocol) and another subject had missing data due to equipment malfunction; therefore, 21 participants had data which was considered ‘complete’ and analyzed. Of these 21 participants, 10 received active stimulation and 11 received sham stimulation.
Subject demographics are detailed in Table 1. Notably, males were overrepresented in the study (15 of 21 participants), which could be attributable to higher levels of caution among females with OUDs, as gender-based violence is an unfortunately common experience among this population [53]. It is, however, important to note that rates of OUDs among women are higher than those among men [53]. Table 2 describes anthropometric information per device group. It is interesting to note that height significantly different between active and sham groups. The study did not control for this factor, as controlling for subject demographic information would have compromised the randomization and blinding of the study.
<table>
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<th>Sex [F/M]</th>
<th>Height [cm]</th>
<th>Weight [kg]</th>
<th>BMI [kg/m²]</th>
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Table 1: Anthropometric information per participant. F, female; M, male; BMI, body mass index.

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Table 2: Anthropometric information and physiological parameter information per device group. P: p-value for the comparison of participants’ characteristics between groups, calculated from student’s t-test for normal continuous variables, Wilcoxon rank-sum test for non-normal continuous variables, or chi-squared test for categorical variables. Normality was assessed using Shapiro-Wilk test. Values represent mean (SD). F, female; HR, resting heart rate; BMI, Body Mass Index.
4.2 Data Processing & Analysis

All signal processing was completed in MATLAB (R2020a, Natick, MA). All signal filtering was performed forward and backward utilizing a finite impulse response (FIR) Chebyshev filter.

4.2.1 Surveys: The change in survey response from before stimulation to after stimulation was computed. For COWS and NRS-Pain, which only had two measurements (before and after protocol), the pre-protocol score was subtracted from the post-protocol score to find \( \Delta \text{COWS} \) and \( \Delta \text{NRS-Pain} \). For the surveys that were administered after every protocol block (see Figure 3), the survey response after the second protocol block was used as the ‘baseline,’ and was subtracted from the survey response after the last protocol block to obtain \( \Delta \text{SUDS} \), \( \Delta \text{VAS-Withdrawal} \), \( \Delta \text{VAS-Craving} \), and \( \Delta \text{VAS-Anxiety} \). The survey response after the second protocol block was chosen because it was the last survey administration prior to beginning stimulation.

4.2.2 Electrocardiogram (ECG): ECG signals were processed as described in previous work [8]. The ECG signals were first filtered with a passband of 0.6-40Hz and then assessed for signal quality; sections of signal determined to be of low quality were removed from further assessment. ECG R-peaks were detected using the Pan-Thomkins algorithm [54], and R-R intervals were determined by subtracting consecutive ‘clean’ R-peak timings. These R-R intervals were then used to determine instantaneous heart rate.

Heart rate variability (HRV) metrics were extracted from the instantaneous heart rate values. Utilizing the PhysioNet Cardiovascular Signal Toolbox [55], HRV metrics including the Root Mean Square of Standard Deviation (RMSSD), the percentage of differences in successive normal sinus intervals which are over 50ms (pNN50) [56], and
Low Frequency to High Frequency ratio (LFHF) were extracted. These particular HRV metrics were extracted due to their utility as metrics of sympathetic tone [57]. Respiratory Sinus Arrhythmia (RSA) was also extracted utilizing the Porges-Bohrer method [58].

4.2.3: Photoplethysmography (PPG): PPG signals were processed as described in previous work [12]. PPG signals were first filtered with a passband of 0.4-8Hz. The PPG waveform was then segmented into beats using the R-R intervals extracted in 4.2.2. The individual beats were then assessed for quality utilizing several techniques. The first step excluded all beats with amplitudes greater than ±5 median absolute deviations (MADs). The second step was to use a 30-beat moving window outlier removal utilizing ±5 MADs to exclude data. Next, a template matching approach was used with an SQI threshold of 0.6 [59]. A new template was then formed using Woody’s algorithm for a second stage of filtering with thresholds of 0.35-0.7. Pulse arrival time was then extracted from this final PPG waveform using the intersecting tangents method [60]. Utilizing another two-stage outlier removal algorithm, PATs that were outside of ±5 MADs were excluded; the final PAT values were then extracted utilizing the intersecting tangents method.

4.2.4 Seismocardiogram (SCG): SCG signals were processed as described in previous work [12]. SCG signals were assessed for quality using methods similar to 4.2.3. First, the signal was filtered non-causally utilizing a Chebyshev FIR filter with a passband of 2-39Hz. The SCG waveform was then segmented into beats utilizing the R-R intervals previously extracted. Any beats with amplitudes outside of ±5 MADs were then excluded. A 30-beat moving window outlier then excluded all beats outside of ±5
MADs. Next, a template-matching approach was utilized, using a 30-second moving window template [61]. Essentially, each waveform was compared to a template formed by all beats within the previous 30s. All beats with a signal quality index greater than 0.53 were retained, then used to form another template [62]. The beats were assessed for signal quality again using methods outlined in [12] to form a preliminary set of SCG beats. PEP was extracted from each beat in this preliminary set utilizing a simplified consistent peak tracking algorithm [63]. These PEP values were used for a two-stage outlier-based beat removal with ±5 MAD thresholds. Finally, the PEP values were extracted from the remaining beats. For all beats which had a both a PEP and PAT value, PTT was extracted by subtracting PEP from PAT. 1/PTT was calculated by taking the reciprocal of the PTT value.

4.2.5 Respiration Belt: The respiration belt signals were processed as described in previous work [8]. The respiration signal was first linearly resampled to 50Hz utilizing an antialiasing lowpass filter, and then bandpass filtered to 0.1—0.72 Hz. Adaptive thresholding, with 60-second windows and 2-second overlaps, was applied to determine peaks. The thresholding enforced a minimum peak prominence of 0.5 of the standard deviation of the data in the window; the minimum time between peaks was set to 1.4 seconds [64]. Respiration onsets were found by using these peaks and locating local minimum between each peak. Therefore, each breath had a corresponding maxima and minima associated with it.

Two different methods were employed to determine quality of data [65]. The first was a power spectral density-based respiration quality index. The filtered data was split into 16 second non-overlapping windows, and then power spectral density was calculated
using a 64-point fast Fourier transform which returned a vector corresponding to the power within frequency bins of resolution 1/16 Hz.

Once respiration quality index was determined, all bins with a quality index greater than 0.45 were retained. Any inter-breath-interval (time between breaths) greater than 10 seconds or less than 1.4 seconds were also deemed physiologically impossible and were removed from the data set. Since each breath had a maximum and minimum identified, inspiration time was calculated as the time from the local minimum to local maximum per breath; the expiration time was calculated as the time from the local maximum to local minimum per breath. The inter-beat-intervals were calculated by taking the reciprocal of time difference between the respiration onsets and multiplying it by 60. For inspiration time, expiration time, and respiration rate, all data points lying outside of ± 4 MADs were flagged. Then, a 30-element moving window outlier was applied to all three respiratory biomarkers; all elements outside of the ± 4 MAD threshold within this window were also flagged. Finally, all onset times and inter beat intervals corresponding to the flagged indices were removed; these final sets of inter beat intervals and onset times were used to extract a final set of respiratory rate, inspiration time, and expiration time metrics.

4.3 Statistical Analysis

For all statistical tests, the group classification (active or sham) was used as the independent variable, and the dependent variable was the biomarker or survey response of interest. Normality of survey groups was assessed via the Shapiro-Wilk test; normally
distributed survey responses were compared via an unpaired t-test while nonnormally distributed responses were compared via a Mann-Whitney U test.

For all biomarkers, the average value during the second control video (protocol block two) was used as baseline. Normality of the values was assessed using the Shapiro-Wilk test; for all normally distributed sets of values, the mean was used, while for nonnormal sets of values, the median was used to determine the baseline biomarker value. The biomarker value during each stimulation block (protocol blocks 3 through 8) was similarly calculated, using the Shapiro-Wilk test to determine whether the set of data points was normally or nonnormally distributed and using the mean or median accordingly. Δ values were determined by subtracting the baseline values from each stimulation block value; this produced six Δ values per biomarker. The mean of these six Δ values was taken to find one Δ value per subject per biomarker.

For each biomarker set, active and sham values were compared. Normality of the biomarker data in active/sham groups was assessed via the Shapiro-Wilk test; normally distributed sets were compared via an unpaired t-test while nonnormally distributed sets were compared via a Mann-Whitney U test. Any p value under 0.1 was considered statistically significant.

4.4 Results

4.4.1 Survey Results: Several of the surveys had significant differences between the active and sham groups (See Figure 6). Namely, ΔNRS Pain, ΔSUDS, and ΔVAS-Withdrawal all had p-values under 0.05. The active group had ΔSUDS of -17.5 ± 26.5
points to the sham group’s ΔSUDS of 2.5 ± 16.9 points for a p value of 0.018; the active group had a ΔNRS Pain of -0.78 ± 2.44 points to the sham group’s ΔNRS Pain of 0.91 ± 1.04 points for a p value of 0.037 (note that one active subject’s ΔNRS Pain score was excluded due to missing data); the active group had a ΔVAS-Withdrawal of -1.90 ± 3.70 points to the sham group’s 1.00 ± 2.68 for a p value of 0.036.

**Figure 6: Survey results for all subjective survey scores.** The top row (NRS-Pain, SUDS, and VAS-Withdrawal) all have significant p values (less than 0.1) while the bottom row (COWS, VAS-Craving, and VAS-Anxiety) all have insignificant p values (greater than 0.1).

Three of the surveys did not show a significant difference between active and sham groups, though all Δ survey results were on average lower in the active groups as
compared to the sham groups (See Figure 6). The active group had a △COWS of 0.60 ± 3.53 points to the sham group’s 1.82 ± 4.07 points for a p value of 0.475; the active group had a △VAS-Craving of -2.20 ± 3.58 points to the sham group’s 0.091 ± 2.70 points for a p value of 0.112; the active group had a △VAS-Anxiety of -1.90 ± 3.45 points to the sham group’s -0.091 ± 1.76 points for a p value of 0.179.

In summary, a significant difference in distress, pain, and withdrawal symptoms were found when comparing active to sham stimulation. Meanwhile, a nonsignificant difference was found in craving, anxiety, and COWS when comparing active to sham stimulation.

4.4.2 Cardiovascular Biomarker Results: Several cardiovascular biomarkers showed significant differences between active and sham groups. The first is heart rate (see Figure 7); the active stimulation group had a △ heart rate of -3.83 ± 2.83 bpm, while the sham stimulation group had a △ heart rate of -0.76 ± 3.49 bpm for a p value of 0.0407. Only one of the heart rate variability metrics showed a significant difference between the active and sham groups, however. The △ ratio of low frequency to high frequency content in ECG (LFHF Ratio) (see Figure 7) was -1.51 ± 2.04 in the active group and -0.22 ± 1.49 in the sham group for a p value of 0.0845. The final cardiovascular biomarker which demonstrated significant difference between the active and sham groups was the inverse of pulse transit time. The △ inverse of pulse transit time was -0.013 ± 0.076 seconds⁻¹ for the active group and -0.23 ± 0.292 seconds⁻¹ for the sham group, for a p value of 0.0372.
Figure 7: Δ heart rate, Δ low frequency to high frequency content of the ECG (LFHF ECG) ratio and Δ inverse of the pulse transit time with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. All three biomarkers show significant differences between the active and sham stimulation groups. Heart rate and LFHF ratio were both lower in the active than the sham group; the inverse of pulse transit time was higher in the active than the sham group.

There were cardiovascular biomarkers that did not show a significant difference between active and sham groups, however. These included Δ pulse arrival time, Δ PPG amplitude, and Δ pre-ejection period (see Figure 8). Δ PAT for the active group was $2.37 \times 10^{-4} \pm 3.8 \times 10^{-3}$ seconds and was $2.3 \times 10^{-3} \pm 8.1 \times 10^{-3}$ seconds for the sham group, resulting in a p value of 0.86. ΔPEP for the active group was $-7.51 \times 10^{-4} \pm 2.2 \times 10^{-3}$ seconds and was $-2.4 \times 10^{-3} \pm 9 \times 10^{-3}$ seconds for the sham group for a p value of 0.833. The Δ PPG amplitude was $-0.99 \pm 1.40$ for the active group and $-0.20 \pm 1.16$ for the sham group for a p value of 0.275.
Figure 8: Δ pulse arrival time (PAT), Δ pre-ejection period (PEP), and Δ PPG amplitude with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. None of the biomarkers show significant differences between the active and sham stimulation groups.

Several heart rate variability metrics also did not show significant differences between active and sham stimulation groups (see Figure 9). These include Δ pNN50 active, which showed an average change of 0.020 ± 0.0344% for the active group, and 0.0037 ± 0.11% for the sham group, for a p value of 0.657. Root mean square of standard deviation (RMSSD) also showed nonsignificant changes in active versus sham stimulation groups. Specifically, the active group had a Δ RMSSD value of 2.23 ± 4.50 ms while the sham group had a Δ RMSSD value of 3.40 ± 13.08 ms for a p value of 0.79. Finally, respiratory sinus arrhythmia (RSA) had a value of 0.15 ± 0.46 ms for the active group and 0.15 ± 0.68 ms for the sham group, for a p value of 0.699.
4.4.4 Respiratory Biomarker Results: Respiratory biomarkers including inhalation time and inhalation to expiration time ratio showed significant differences between the active and sham groups (See Figure 10). \( \Delta \) inhalation time was \(-0.46 \pm 0.43\) seconds for the active group and \(-0.046 \pm 0.27\) seconds for the sham group, for a p value of 0.0159. The \( \Delta \) inhalation to exhalation time ratio was \(-0.22 \pm 0.33\) for the active group and \(-0.0069 \pm 0.16\) for the sham group, for a p value of 0.076.

Figure 9: \( \Delta \) pNN50, \( \Delta \) root mean square of the standard deviation (RMSSD), and \( \Delta \) respiratory sinus arrhythmia (RSA) with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. None of the biomarkers show significant differences between active and sham groups.
Figure 80: Δ inhalation time and Δ inhalation to exhalation time ratio with p values. Note that each data point corresponds to the average of the six ‘during stimulation’ to ‘baseline’ changes per subject. Both biomarkers show significant differences between active and sham groups; inspiration time and inspiration to expiration time ratio are both lower in the active than sham group.

Interestingly, while inhalation time and ratio of inhalation to exhalation time changed significantly, Δ exhalation time and Δ respiration rate both did not have a significant difference between active and sham groups (see Figure 11). Δ exhalation time was -0.17 ± 0.57 seconds for the active group and 0.011 ± 0.42 seconds for the sham group, for a p value of 0.86. Δ respiration rate in the active group was 1.29 ± 2.12 breaths per minute and in the sham group was 0.23 ± 2.61 breaths per minute for a p value of 0.323.
4.4.5 Results Summary: The change in several cardiovascular, heart rate variability, and respiratory biomarkers, as well as survey responses, demonstrated a significant difference between the active and sham device groups. Heart rate decreased more in the active group than sham group; the low frequency to high frequency ratio of the ECG signal decreased more in the active group than sham group. The inverse of pulse transit time decreased more in the sham than the active group. Inhalation time decreased more in the active than sham group. The ratio of inhalation to exhalation time decreased more in the active than sham group. Subjective surveys demonstrated that the active groups experienced less pain, distress, and withdrawal than sham groups.
Meanwhile, several biomarkers did not show significant differences between the active and sham device groups. These included cardiovascular biomarkers such as pulse arrival time, PPG amplitude, and pre-ejection period, respiratory biomarkers such as exhalation time and respiration rate, and survey responses such as COWS, craving, and anxiety.

4.5 Discussion

A randomized sham-controlled double-blinded clinical trial of transcutaneous cervical vagus nerve stimulation in opioid withdrawal patients showed significant differences in physiological and subjective measures of opioid withdrawal for active and sham participant groups [66]. Specifically, several subjective measures of opioid withdrawal symptoms were reduced by tcVNS, and several physiological measures of withdrawal were altered by tcVNS. Interestingly, not all physiological measures changed in the ways we classically associate with parasympathetic activation and sympathetic inhibition. However, tcVNS was shown to significantly reduce several risk factors of opioid use relapse, which support the use of tcVNS for individuals with Opioid Use Disorder, specifically during detoxification periods prior to medication-assisted treatment, which have inherently higher risks of relapse [67].

4.5.1 Subjective Responses Showed Risk Factor Reduction: tcVNS was shown to reduce several subjective risk factors of opioid relapse, including withdrawal symptoms, pain, and distress. During discontinuation of opioids, all three of these factors significantly contribute to relapse and behaviors with a high risk of overdose death. Specifically, withdrawal symptoms strongly influence OUD patients’ relapse potential,
especially directly after opioid discontinuation [68-71]. In fact, withdrawal symptom avoidance is often given as a reason for continued opioid use among patients [68]. Pain avoidance is another key factor for patients when deciding whether to use opioids; often, patients develop OUDs after their use of prescription opioids for chronic pain [72-74]. In addition, long-term use of opioids often leads to opioid-induced hyperalgesia [75]. This increased sensitivity to pain can lead patients to continue opioid use in order to avoid pain; additionally, if the chronic pain that led to opioid use in the first place is still present, this pain could be worsened by the hyperalgesia and lead to opioid continuation [76]. Stress is another major risk factor of continued opioid use [69, 77, 78]. Many patients cite stress avoidance as a major factor in their OUD, as they use opioids to overcome stressors rather than to ‘get high’ [68, 79]. Stress is often a leading cause of relapse, even after long periods of abstinence [80, 81]. Reduction of withdrawal symptoms, pain, and distress can all help prevent opioid relapse; this provides convincing evidence for use of tcVNS during OUD cessation periods.

It is interesting that COWS, anxiety, and craving symptoms did not show significant differences between active and sham groups. While on average all these scores decreased, there may be some physiological reasons underpinning this discrepancy. Though it may seem contradictory that Δ VAS-Withdrawal showed a significant difference between active and sham groups while Δ COWS did not, this could be due to the fact that Δ VAS-Withdrawal is based on the subject’s own assessment of their withdrawal symptoms, while Δ COWS is based on 11 subjective and objective parameters [51]. If a subject’s self-evaluation of withdrawal symptoms considers only a few factors that the subject considers most important – say, pain and distress – while
COWS considers several other categories, it follows that a change in Δ VAS-Withdrawal could be separate from a change in Δ COWS. It is important to note that a patient’s decision to use opioids after cessation is based on the patient’s assessment of their withdrawal symptoms, not on a holistic view of all withdrawal categories.

It is also interesting to note that though Δ VAS-Craving scores differed from active and sham groups by an average of 2.29 points, this was not found to be a significant difference. It seems intuitive to think that craving, withdrawal, and distress symptoms would all be mediated similarly by tcVNS; however, there are potential physiological explanations underlying the discrepancy. Specifically, craving and withdrawal are mediated by different brain regions. The prefrontal cortex is the main mediator of executive dysfunction, which is the cause of craving [80, 82]. However, withdrawal is associated with dysregulation of emotions and the autonomic nervous system, which is linked to the extended amygdala, hypothalamus, and brainstem regions [80, 82]. These regions are also associated with distress, which similarly showed a significant difference between active and sham groups [83]. This suggests that tcVNS may affect the autonomic and limbic systems more strongly than the prefrontal cortex, which is responsible for executive function; this could explain why craving did not significantly change while distress and withdrawal did. Functional neuroimaging studies of subjects undergoing tcVNS during opioid withdrawal are required to evaluate this hypothesis.

4.5.2 Cardiovascular Biomarkers Demonstrated Mixed Response to tcVNS: While the changes in heart rate and LFHF ratio supported the hypothesis that tcVNS reduces
sympathetic activation as compared to sham stimulation, PTT$^{-1}$ provides conflicting evidence.

On average, $\Delta$ heart rate was 3.07 beats per minute lower in the active group than the sham group. Heart rate is controlled by both branches of the autonomic nervous system; a decrease can be attributed to an increase in parasympathetic activity and/or a decrease in sympathetic activity [34]. This result supports the hypothesis that tcVNS dampens sympathetic activity and increases parasympathetic activity.

$\Delta$ LFHF ratio was on average 1.31 AU lower in the active group than in the sham group. We know that low frequency content of the ECG signal is typically associated with sympathetic activity, while high frequency content of the ECG signal is associated with parasympathetic activity [35]. Therefore, a lower LFHF ratio would be associated with lower sympathetic activity and/or higher parasympathetic activity; this supports our hypothesis that tcVNS dampens sympathetic and heightens parasympathetic activity.

$\Delta$ PTT$^{-1}$ was on average 0.217 seconds$^{-1}$ higher in the active group than in the sham group. This is contrary to what we expect during tcVNS; in fact, previous experiments on patients with post-traumatic stress disorder (PTSD) have shown that tcVNS lowered $\Delta$ PTT$^{-1}$ [12]. PTT$^{-1}$ is directly related to blood pressure and can be used as a noninvasive wearable corollary for continuous blood pressure monitoring. As sympathetic arousal is related to a higher blood pressure, we would expect sympathetic dampening to lower blood pressure and therefore lower PTT$^{-1}$ [12]. This result suggests that there are potentially physiological differences between OUD patients and PTSD patients that effect blood pressure and patient response to tcVNS stimulation.
4.5.3 Respiratory Response Dominated by Change in Inhalation Time: Inhalation time decreased during tcVNS as compared to sham stimulation by approximately 0.41 seconds; during inhalation, sympathetic activity increases relative to parasympathetic. That is, vagus activity is higher during exhalation than inhalation. Therefore, it makes sense that the inhalation period was shortened – this means that there is a shorter period of time during each respiratory cycle that the sympathetic nervous system is higher relative to the parasympathetic nervous system. However, it is interesting that exhalation time was not changed by tcVNS, as exhalation time is associated with vagal activity and an increase in parasympathetic activity relative to sympathetic activity.

The ratio of inhalation to exhalation time also significantly decreased in the active stimulation group as compared to the sham stimulation group by 0.21 AU. Again, since the exhalation period is associated with increased vagus and parasympathetic activity, a lower ratio of inhalation to exhalation time corresponds to more parasympathetic activity relative to sympathetic activity, which is ultimately the goal of tcVNS [39]. It is important to note that the majority of this change is due to the change in inhalation time rather than change in exhalation time. However, exhalation time has been associated with vagus nerve activity; perhaps this suggests that tcVNS works at least in part by decreasing sympathetic tone rather than increasing parasympathetic and overall vagus activity.
CHAPTER 5

CONCLUSION & FUTURE WORK

5.1 Conclusion

This study is the first sham-controlled, randomized study of non-invasive neuromodulation in human subjects undergoing active opioid withdrawal [66, 84]. The observed differences in distress, withdrawal, and pain provide evidence to support the efficacy of tcVNS for preventing relapse risk factors among OUD patients. Additionally, the reductions in heart rate, LFHF ratio, and inspiration time all support the hypothesis that tcVNS dampens sympathetic activity and increases parasympathetic activity. However, the change in Δ PTT⁻¹ provides counterevidence to this claim; it is interesting to note that this metric is affected by both blood pressure and vascular tone.

tcVNS shows potential as a treatment for OUD and withdrawal symptoms, and provides a non-invasive, minimally risky, non-pharmacological treatment for a period when patients are unable to seek any other forms of treatment – the detoxification period before patients can begin medication assisted treatment. OUD patients beginning abstinence or medication-assisted treatment are at a heightened risk of overdose death and targeting this time could provide treatment options for a period when patients are vulnerable. tcVNS is also easily self-administered and provides a non-pharmacological option for this withdrawal period. Though more research should be conducted, tcVNS shows initial promise for reducing relapse risk factors; if further studies provide evidence to support reductions in relapse and/or overdose rates, this therapy could quite literally save thousands of lives.
5.2 Limitations

Limitations of this study include sample size, potential confounding factors, and data collection location. The sample size of this study was relatively low at 21 participants; this is due to the challenges with recruitment, retention, and data collection. Working with patients undergoing active opioid withdrawal poses several challenges that will need to be overcome to perform a study with a larger sample size. There were potential confounding factors between the active and sham groups, namely height and gender; however, controlling for these factors would compromise the randomization of the study. The study was also conducted at two separate study locations, and so data collection environment was not standardized for patients. However, there was no significant difference between the data taken from patients at the two sites.

5.3 Future Work

5.3.1: Future Work Utilizing this Data Set: There are several ways to utilize this data set more thoroughly than has been done. The first step would be to analyze the signals that have not yet been analyzed: electrodermal activity (EDA), impedance pneumography (IP), and blood pressure. IP can provide more information on respiration volume, timings, and variability. IP can supplement the respiration belt data, especially when respiration belt data was ignored due to a noisy signal. EDA and blood pressure could provide more metrics of sympathetic activity, potentially strengthening the hypothesis that tcVNS dampens sympathetic activity in OUD patients.

There are also several ways to leverage the processed dataset to learn more about how tcVNS affects the sympathetic nervous system. For example, PPG amplitude
change after stimulation could be compared between sham and active stimulation groups to better align with the methods in past literature [85]. In fact, no time points post-stimulation have been examined for this data set; it would be interesting to learn more about the time-course of stimulation and whether the autonomic effects of stimulation last past the stimulation period. Additionally, instead of using a baseline during the neutral videos, more acute stimulation effects could be examined by comparing the change in biomarkers from the period directly preceding stimulation to during stimulation. There are also more measures of sympathetic activity that can be extracted from signals such as the seismocardiogram and ECG; the biomarkers extracted herein are only a sampling of what can be extracted from the data.

5.3.2: Future Work Building upon this Trial: There are several ways to build upon this study to determine whether tcVNS is an effective treatment for OUD patients undergoing active withdrawal and what effects tcVNS has on OUD patients. Longitudinal studies which track relapse rates among OUD patients utilizing tcVNS could be used to learn whether tcVNS can affect relapse and/or overdose rates. Future studies should utilize functional neuroimaging during tcVNS to determine if there is a difference in brain activity during tcVNS as compared to sham; this can also provide evidence to prove or disprove the theory that brain areas like the extended amygdala, hypothalamus, and brainstem are affected more by tcVNS than is the prefrontal cortex. Blood samples could be added to future studies to examine blood biomarkers such as interleukin 1B, tumor necrosis factor, and interferon-gamma, as past studies have shown changes in blood biomarkers in different patient populations [4]. Additionally,
continuous blood pressure monitoring should be explored, as correlates of blood pressure showed counterintuitive trends during this study.
REFERENCES


