NOVEL TECHNOLOGIES FOR THE DIAGNOSIS AND TREATMENT OF POSTTRAUMATIC STRESS DISORDER

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NOVEL TECHNOLOGIES FOR THE DIAGNOSIS AND TREATMENT OF POSTTRAUMATIC STRESS DISORDER

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To my mothers, Zerrin and Nesrin, and my grandmother, Saimoş, for their love that will last forever.

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One day, after a somewhat impulsive decision, I told my family that I was heading to Seattle for an exchange program, to help me decide if the United States was worth it for grad school (and to check out the land of grunge). I did not ask for their permission, I only let them know, just like with every other thing I do. For instance, I traveled Europe with friends many times when I was a teenager, only informing my family that I was leaving next week to this or that country. Though they worried, my family largely accepted my independent nature.

Then, though, I consciously selected a difficult male-dominated career path: engineering. I was at my family's limit at that point. To them, it would be more reasonable for me to go to medical school, which would mean a "better" future for me given Turkey's circumstances and I would not be "wasting" my university preparation struggle. My father, an engineer himself, warned me, in one of the most serious conversations I have had with him, that I would have to work harder than my male colleagues to fit in as an engineer. He worried that I would end up damaged, tired, stressed, demoralized. My uncle, a professor of pathology, shared his reason to study veterinary medicine rather than medicine - his pigeon that died in his hands when he was a young boy. He told me that he understood me and my passion, but his own impulse to study veterinary medicine had not been worth it: every animal has a different physiology, he would have better exceled at treating humans rather than different animals, and medicine would have been a better career path in Turkey. My mother, who always wanted to become a pediatrician but ended up as an English Literature major, begged me to go to medical school. Because she is undeniably smarter

than me, she also tried psychological manipulation through my friends, especially Hande Kucukunsal, and the subtle tactic of us later we ended up watching Pathology. Elegant try, canım annem.

I refused to be dissuaded. I pulled off on my own. I started a summer job at a call center right after the university entrance exam, with night shifts, so as to not confront my mother during day hours. I had *always* wanted to be an engineer. Nothing and no one could change that simpy because I did well at a ridiculous university entrance exam that haunts Turkish youth with lifelong traumas.

I am forever grateful to my family for loving me while letting me live my independence, and also for raising me without any kind of tribalist conditioning. Growing up in Istanbul, which is bigger and more populous than any city in the United States, particularly having been raised in a historically Armenian minority district, where you can hear both the church bells and mosque's call to prayer, certainly helped to prepare me to spread my wings in different cultures (also, potentially, my alleged Greek/Russian heritage, according to some internal family musings).

Many thanks to my family and friends for tolerating me while I struggled for years working on my PhD. I apologize to my family for being inaccessible, especially during these pandemic days when I have been writing my dissertation and finishing up my PhD work. Them and their health were on my mind everyday.

During my studies in the United States, I have had many happy times, dodged a few bullets, and made many mistakes, including but not limited to traffic accidents. My family and friends know only a few, aside from the automotive accidents. I do not have terrible regrets.

I am truly grateful for the memories, experiences, and the people I met from all walks of my life. I am neither good at nor a big fan of inspirational messages, so hopefully this will not turn into one.

I owe a great deal to my advisor, Omer Inan. I was an undergraduate student at Bogazici Electrical and Electronics Engineering when we first met in 2014, sometime after my first PhD application to Georgia Tech. I always felt that my interview with him went well, but I was not selected. A bizarre cascade of events, though, later led me to Georgia Tech after my first PhD attempt (and after finally getting accepted in 2016), and I have been truly blessed to work with him. I am not a "meant to be" person, but I cannot deny that working with Omer was truly meant to be for me. My coming to Georgia Tech was followed by me requesting to change my PhD research from heart disease to PTSD. Omer did not hesitate to accept, although he knew it would mean a ton of work, deadlines, DARPA reports, and teleconferences.

Just like my family, Omer gave me independence. He always had a positive and supportive attitude to every e-mail I sent, no matter how crazy it could sound. I was free to make collaborations and work on additional publications that were not entirely relevant to my PhD work with him. Before PhD, I did not imagine that independence would be a requirement for me in my career path. Independence is now a requirement for me, partly because Omer and others supported it when helping me to flourish. Omer was also the first person to know about the hiring freezes after each of my interviews took place and helped me through my pessimism during COVID-19 times.

My time in Atlanta was substantially enriched by meeting my Emory collaborators: Doug Bremner, Viola Vaccarino, Amit Shah, Matt Wittbrodt, Brad Pearce, Emily Driggers, Stacy Ladd, Raj Dedhia, Jeanie Park, Shawn Hochman, Heidi Kloefkorn, and many others. As a film buff and activist, I could never have imagined having a PI who is also a film director as well as an activist, one who also happened to be the expert witness for a lawsuit regarding a pharmaceutical I used in my high school years. Filled with Doug's creative personality, Viola's vision, Amit's support for my first paper rejections, Matt's rigorous critiques, Brad's immunology guidance, Shawn and Heidi's physiology and stress expertise, and the rest of the Emory team's help in clinical research, our work together was an enjoyable feast for me. In no particular order, I would like to thank Steven Rhodes, Nancy Murrah, Margie Jones, Lucy Shallenberger, Emily Driggers, Jamil Alkhalf, Yunshen Jiao, Allison Hankus, Puja Mehta, Oleksiy Levantsevych, Yi-An Ko, Ernest Garcia, Minxuan Huang, Jonathon Nye, Bruno Lima, John Nye, Joy Beckwith, Lucy Shallenberger, Hewon Jung, Mobashir Shandhi, Asim Gazi, and Hyeon Ki Jeong for assisting me in clinical and preclinical research and being ready with me at the Wesley Woods Hospital, Emory Brain Center, and Hochman Lab for every patient or every mouse model with the same enthusiasm and dedication. Gaining this sort of expertise is a huge bonus for an ECE PhD student. Outside of Emory, I want to thank Mozziyar Etemadi, Radha Poovendran, Chris Rudell, Yasemin Kahya, Raj Dedhia, Mani Soma, and Lih Lin for supporting and encouraging me at different phases of my academic training. I also greatly appreciate my PhD committee members Robert Butera, Javier Hernandez, Hua Wang, and Doug Bremner for their time and guidance. I thank Javier Hernandez who not only accepted to serve in my committee after a 20-minute meeting I had with him over a

very short Boston visit, but also guided me through my attempts to realize the final aim of my PhD dissertation.

My research could not have been done without the support and encouragement of Georgia Tech and Emory University faculty, and the interdisciplinary ecosystem these two top notch institutions provided. I am grateful to Magnus Egerstedt, Azadeh Ensari, Pamela Bhatti, Daniela Staiculescu, Alenka Zajic, Chris Rozell, Gregory Abowd, Rosa Arrriaga, Rozga Agata, Jackie Nemeth, Ashlee Gardner, Leyla Conrad, UbiComp Group researchers Vedant Das Swain, Mehrab Morshed, and current and past Georgia Tech friends Sinan Hersek, Asim Gazi, Beren Semiz, Maziyar Baran Pouyan, Dotun Ode, Mobashir Shandhi, Hewon Jung, Mohsen Safaei, Nordine Sebkhi, Samer Mabrouk, Hyeon Ki Jeong, Venu Ganti, Jacob Kimball, Jon Zia, Brandi Nevius, Sevda Gharehbaghi, Kristy Scott, Goktug Cihan Ozmen, Hazar Ashouri, Jordan Conant, Abdul Qadir Javaid, Andrew Carek, Caitlin Teague, Lara Orlandic, Yaoyao Jia, Nazli Goller, Nick Bolus, Daniel Whittingslow, Shafaat Ali Sheikh, Jeffrey Ward, Alexis Dorier. I am forever grateful for future faculty workshops and practicums the School of ECE faculty provided that prepared me for the academic job market. All faculty hire positions I interviewed for have been suspended due to COVID-19, but I am hopeful that these experiences will bear fruit in coming years.

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Besides my Maryland family, I also have a Seattle family. Many thanks to Brenda Donner and Bruce Brewer for welcoming me in my first days in Seattle in 2012. I met them during a two week period designed as a warm introduction to "American life" by the University of Washington's International Students Office when I was an undergraduate exchange student. We have never disconnected. Brenda always has remembered me whenever there is a political turbulence in Turkey (indeed, it is hard to find a time frame there is *not* any sort of turbulence in Turkey, so we have communicated quite frequently). Her considerate messages made me aware how empathetic she was toward a student she barely saw a few days; I hope to model her caring.

I also have an Atlanta family, who have been a blessing to me. One of the most pleasant surprises from my time at Georgia Tech has been having the pleasure to meet and bond with the Milor family. Vedat Milor, who is a food & wine critic I admired for years, happened to live in Atlanta with his wife Linda, a professor in my department, and his daughter Ceylan. Interestingly enough, I shared two other schools with them: Vedat was a Bogazici graduate and Linda worked at the University of Maryland College Park many years ago. Vedat's admirable search for beauty in everything, Linda's concise and effective career advice, and Ceylan's curious eyes that brighten to every strange thing she encounters, including salep and New York cheesecake, made my time in Atlanta much more memorable. I especially thank my youngest friend Ceylan for improving my *Turklish*, reminding me of my Turkish song playlists on Spotify which I had long neglected, making me discover the existence of Bluetooth AUX receivers as an electrical engineer, and looking amazed at every Turkish food recipe I tried (they were just primitive imitations of my mother's recipes).

Last but not least, I would like to thank my friends who always adjust their already busy schedules to make time for me whenever I go back to Istanbul: Hande Kucukunsal, Sezen Sari, Begum Konuralp, Sevgi Gokce Ceran, Seda Bakan, Arzu Takil, Gizem Karakadi, Ozlem Pinar Akin, Ilhan Yildirim, Dilan Ecem Karakaya, Bilge Ozata, Sinan Can Okutan, and others. Sorting by geographical location, I thank my friends in the United States (Burak Nehbit, Nilsu Goren, Besim Halit Yasar, Anshuman Sinha, Maya Kabkab, Berk Gurakan, Kleoniki Vlachou, Bige Deniz Unluturk, Suna Pelin French, Brett Murphy, Seyma Gurkan, Ayse Selin Cakmak, Fatma Karagoz, Sezen Yucel, Can Sever, Idil Arsik, Selcuk Karakas, Lina Castano), in Europe (Ece Gulay, Asya Cubuk, Samy Halaji), and friends/family who could be anywhere in the world anytime (Gozde Tutuncuoglu, Gamze Akarsu, Aysun Unal, Ecem Unal, Eren Unal, Ercument Unal, Esen Moya). I owe so much to my dear friend Brett Murphy, for his tireless reading of my many drafts (including this one), and his many useful suggestions. I am lucky to have this incredible social support network.

There are a few events I wish I had not missed, for example, Ece's Zagreb Wedding, and Gizem's and Gokce's Istanbul Wedding. After my PhD, I will try to not miss these happy moments of my friends, no matter where in the world they happen. I am looking forward to traveling for them more often.

Where you live becomes your boundary. I am fortunate to have lived in many places and had my boundaries expanded across the oceans. Many thanks to all my friends, family, mentors, mentees, collaborators, reviewers, colleagues for my training and development as a human being and a researcher.

People in need in Turkey and the United States are not terribly different. Here under the umbrella of racism and poverty, there under domestic abuse, lack of freedom, and poverty. It is not hard to map the problems of one place onto another; the reality is the people need help. You can volunteer, you can work for them for free. You will not be able to cause a significant change unless you educate the most vulnerable ones, re-defining reality in their eyes, and inspire them to stand up for themselves and others.

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SUMMARY

The brain and the heart share an active and reciprocal dialogue, continuously modulating each other's function. For individuals who have experienced traumatic events, the reminders of these events affect both the brain and heart due to this intimate relationship, and can later develop into posttraumatic stress disorder (PTSD) due to the repeated activation of trauma-related neuropathways and autonomic imbalance. Electrical stimulation of the vagus nerve —the longest cranial nerve, which regulates the autonomic state—using an implantable device is a potential treatment method to address such imbalance. Noninvasive vagal nerve stimulation (nVNS) devices offer inexpensive and low-risk alternatives to surgical implants, but their effects on the physiology are not well understood. Real-time, noninvasively obtained biomarkers are required to tailor therapy and to close the loop for automated delivery.

This dissertation focuses on identifying and developing noninvasive technologies for nVNS in the context of PTSD. Identification of noninvasive measures that can diagnose and treat PTSD is imperative for at-home usage and for developing closed-loop systems. This research first focuses on how noninvasive sensing modalities could be instrumented and used in conjunction with signal processing and machine learning methods to quantify an individual’s autonomic state. Second, a mechanistic, sham-controlled, randomized, double blind study on the use of nVNS for dampening stress response is investigated in multiple dimensions: downstream physiological effects and biochemical biomarkers, with a particular focus on real-time physiological biomarkers and their potential for closing the loop for machine learning guided personalized neuromodulation. The broader impacts of

this research cover accessible, low-cost diagnosis and treatment options for patients with stress-related neuropsychiatric disorders, which are important public health problems and projected to increase due to COVID-19 pandemic. The sensing modalities, algorithms, biomarkers, and methodologies detailed in this dissertation lay the groundwork for future efforts to objectively diagnose and treat neuropsychiatric disorders remotely, outside of clinical settings.

CHAPTER 1. INTRODUCTION

The brain and the heart share an active and reciprocal dialogue, continuously modulating each other's function. For individuals who have experienced traumatic events, the reminders of these events affect both the brain and heart acutely and longitudinally due to this intimate relationship, and can later develop into stress-related psychiatric disorders, such as posttraumatic stress disorder (PTSD), and the autonomic imbalance associated with it. The standard of care for PTSD includes psychotherapy and/or pharmaceuticals, and there is insufficient evidence supporting their efficacy. Neuromodulation is a broad name given to non-pharmacological methods involving the delivery of energy to the body to modulate nerve activity. Electrical neuromodulation methods –also termed as "electroceuticals" – use electricity for this modulation. Artificial electrical stimulation of the vagus nerve–the longest peripheral nerve, which regulates the autonomic state–using an implanted device (vagus nerve stimulation, VNS) is a potential treatment method to address such autonomic imbalance. This dissertation focuses on noninvasive VNS– the low cost and non-surgical alternative of VNS– in the context of stress and PTSD. Specifically, this dissertation details the understanding, identification, development, and validation of biomarkers and technologies that could quantify the stimulation and mitigate the human body's stress response. These inexpensive and potentially ubiquitous technologies could help manage stress and stress-related psychiatric disorders at home and improve the quality of life for millions of patients.

1.1 Motivation

Lasting disruptions of peripheral autonomic and cardiovascular function after exposure to extreme traumas such as the stress of war are best thought of from larger developmental and evolutionary perspectives [2]. Behaviors such as hypervigilance, hyperarousal and light sleep that had survival value for primitive hunter gatherers thousands of years ago are no longer useful for humans who no longer have natural predators [3]. Symptoms of anxiety probably served specific purposes: for instance, if there was a pack of lions in the area where a band of hunter-gatherers was camped, this could lead to an increase in fear and vigilance, which may have made the difference between life and death. These symptoms would persist for long periods of time, possibly after the threat had been removed. The fight or flight response that was formerly critical to survival holds less importance in the modern age when many people in developed countries are rarely under actual physical threat. Activation of the fight or flight response involves the peripheral autonomic and cardiovascular systems, with the resultant symptoms of rapid heart rate and respiratory changes. If these responses become chronic, they can lead to the development of cardiovascular disease, or acceleration of the disease process [4]. Similarly, in combat arenas soldiers develop behaviors like being easily startled that may have survival value especially if there is an ongoing threat of unexpected attack. It is only when the soldier returns from the battlefield and is unable to turn off the "combat mind" that he or she runs into trouble. Behaviors that were effective and necessary in one environment suddenly become not so in another. Chronic stress not adaptive in modern contexts can therefore lead to psychiatric symptoms in some individuals, in addition to accelerating the progression of cardiovascular disease [4]. Due to this close relationship between the heart and mind dynamics, Da Costa's observations on "Soldier's Heart Syndrome" describing

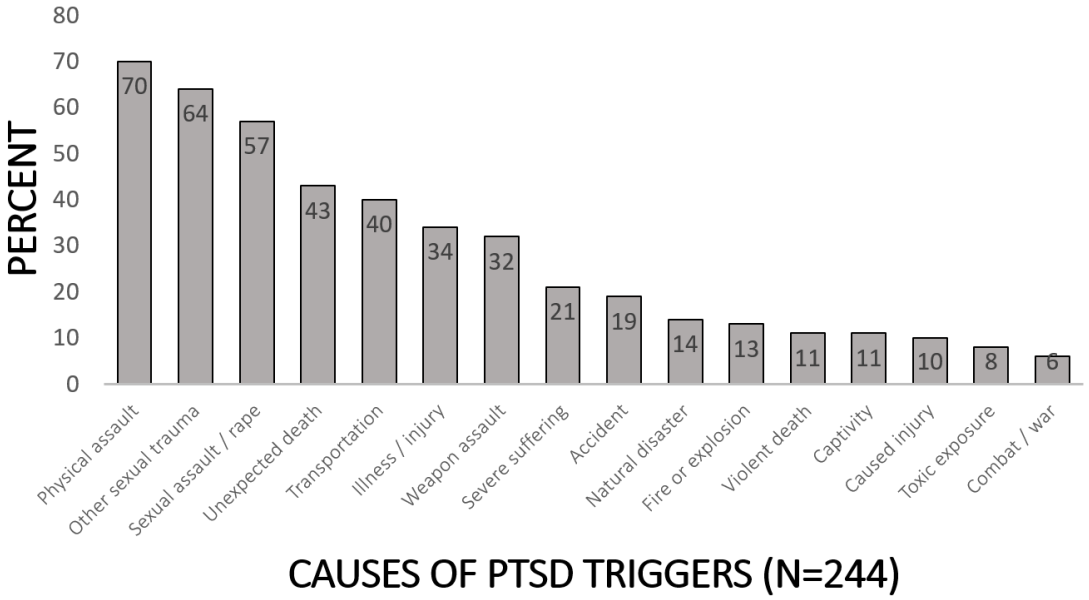

Figure 1-1. Causes of PTSD triggers, adapted from [13]. One individual might have experienced multiple traumatic events.

how soldiers following exposure to combat develop an array of symptoms including fatigue, shortness of breath, sighing respirations, palpitations, rapid heart rate, and sweating [5] still holds value today under the name "Posttraumatic Stress Disorder (PTSD)" [6].

In today's world, Soldier's Heart Syndrome and other conditions such as depression and anxiety have been clinically recognized by the Diagnostic and Statistical Manual of Mental Disorders [7]. PTSD was officially recognized by the American Psychiatric Association in 1980s and was classified as a "mental disorder", unofficially representing a separation of this condition from the rest of the body [8].

Stress-related psychiatric disorders, including depression and PTSD are important public health problems, and unfortunately PTSD is not limited to combat exposure in modern days. Early life stress increases the risk of development of depression in adulthood [9, 10], and stressful life events are associated with an increased risk for depressive episodes [11]

while PTSD requires exposure to a traumatic stressor as part of the diagnosis [12]. Events from sexual assault to accidents, toxic exposure [13], and very likely pandemics such as COVID-19, may produce intrusive memories (Figure 1). The intrusive thoughts of the event may persist in vulnerable individuals and may eventually lead to PTSD. At any given time, 10% of the United States population meets criteria for major depression or other mood disorders based on the Diagnostic and Statistical Manual of Mental Disorders [7] criteria [14], with an annual cost of lost productivity of $44 billion [15], Similarly, PTSD affects 6% of the population at some time in their lives [16]. The cost of treating PTSD and co-morbid depression in soldiers returning from the wars in Iraq and Afghanistan has been estimated to be $6.2 billion dollars [17], and since PTSD affects a larger total number of civilians in the United States than military personnel, the costs for society as a whole are likely much higher [18]. The most common cause of PTSD in women is sexual abuse and assault in childhood, while for men it is physical assault [19]. On average, women have higher occurrence of PTSD compared to men in the civilian population [20, 21]. PTSD is characterized by intrusive thoughts, nightmares, avoidance, emotional blunting, negative cognitions, hypervigilance, and hyperarousal [22]. Depression is associated with depressed mood, loss of appetite, decreased psychomotor activity, and in extreme cases suicidal ideation. Other symptoms, such as poor sleep and concentration, negative cognitions, loss of interest in things and anhedonia, are common to both conditions, and in fact there is a degree of co-morbidity between the two conditions [23-28]. Furthermore, patients with co-morbid disorders have a worse clinical course, with for instance a higher risk of suicidal ideation [29, 30].

The standard of care for PTSD includes psychotherapy and/or medication [31, 32]. Psychotherapy treatments for PTSD, however, have drop-out rates as high as 50%, which limit their applicability [33, 34]. First-line medication treatments for stress-related psychiatric disorders involves the Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants [35, 36]. However, as highlighted by a report from the Institute of Medicine, there is not sufficient evidence to conclude that they are effective for PTSD [37]. In fact, only one third of those suffering from PTSD are able to achieve full remission with the current standard of care [35]. Similar limitations exist for treatment of major depression [38]. Given limitations of current treatment options, new paradigms are clearly needed for the management of stress-related psychiatric disorders.

Besides the limitations and accessibility issues regarding treatment, PTSD diagnosis itself stands as a challenge. PTSD can be difficult to diagnose, because of the subjective nature of most of the diagnostic criteria (similar to other neuropsychiatric disorders), and symptom overlap with other disorders such as obsessive compulsive disorder, generalized anxiety disorder, or substance abuse disorders [39]. There are a number of PTSD screening instruments for adults, such as PTSD Checklist for Diagnostic and Statistical Manual of Mental Disorders 5th Edition (PCL-C for DSM-5) [12] and Clinician Administered PTSD Scale (CAPS) [40], handled as forms of structured interviews by medical professional, lasting approximately for an hour, which makes the accessibility to diagnosis challenging for public. Compared to the United States, there seems to be less research in other countries prone to political or economic turbulence. For instance, the number of research articles indexed at Google Scholar since 2016 in Turkey is 80% lower than the corresponding research in the United States. Despite other factors that might influence research output—

such as number of PTSD researchers or the ecosystem supporting researcher-clinician interactions—, limited numbers of PTSD-related reports are counterintuitive given the rates of sexual, child abuse, political/economic turbulence, and population density. Public stigma on mental health consultation likely adds to the reasons of under-reported PTSD cases [41, 42]. There is clearly a need to accelerate diagnosis and potentially to leverage wearable sensing, without a physical visit to a clinic needed.

This dissertation focuses on technologies to quantify a novel and accessible treatment targeted at patients with PTSD to mitigate the body's fight or flight response and methods to objectively assess PTSD. This potential treatment has also been validated through serum and blood biomarkers and self-reported surveys with a sham-controlled double-blind clinical trial. This dissertation lays the groundwork for techniques to translate this potential treatment from clinics to homes, and methods to objectively diagnose PTSD using only noninvasively measured autonomic nervous system activity information, without the need for clinician administered structured interviews.

1.2 Major Contributions of This Work

While there are potential behavioral therapy-based treatments for PTSD, access to medical professional and side effects of pharmaceuticals limit their applicability. This work focuses on a low cost, noninvasive, and non-pharmacological treatment to electrically stimulate the longest peripheral nerve [43], a key modulator of the autonomic nervous system activity. This treatment have been extensively evaluated in terms of its effects on physiological reactivity, blood and serum biomarkers, behavioral data, and noninvasive biomarkers that could quantify the stimulation presence and efficacy, in patients with PTSD or without

PTSD but with prior trauma exposure as a sham-controlled double blind study. Additionally, based on the physiological data obtained from this human subject group, an objective PTSD assessment method have been developed that only requires recollection of the personal traumatic event. The main contributions of this work are given below:

1. In a sham-controlled, double blind scheme, this work demonstrated that pairing noninvasive vagus nerve stimulation with traumatic reminders in patients with PTSD reduces the fight-or-flight response to emotional triggers and thereby could provide a completely new mode of treatment. This paradigm also reduces stress reactivity to non-personalized mental stress and stress reactivity in human subjects without PTSD.
2. This potential therapy was further validated in acute settings by investigating clinically relevant blood and serum biomarkers and self-reported behavioral outcomes.
3. Utilizing signal processing and machine learning, techniques for measuring and extracting continuous biomarkers of noninvasive vagus nerve stimulation based on non-standard physiological sensing modalities quantifying autonomic reactivity were designed and validated.
4. Leveraging the continuous autonomic biomarkers, a machine learning-guided target engagement quantification method was developed based on fusing multiple modalities that can be incorporated in wearable technology.
5. An autonomic reactivity based, objective PTSD assessment scheme was developed that predicts the PTSD status, for which the state-of-the-art diagnosis methods rely on clinician administered structured interviews and self-reports.

1.3 Dissertation Organization

The rest of this dissertation is organized as follows: Chapter 2 discusses the physiological background of stress and PTSD. Chapter 3 discusses noninvasive sensing and signal processing methods to quantify autonomic reactivity. Chapter 4 presents the work related to noninvasive vagus nerve stimulation in PTSD and non-PTSD human subjects. Chapter 5 introduces an autonomic reactivity based PTSD diagnosis scheme that only relies on objective measures such as physiological stress reactivity, demographics, and background. Lastly, Chapter 6 concludes this work and provides directions for future work.

CHAPTER 2. PHYSIOLOGICAL AND CLINICAL BACKGROUND

Stress is associated with activation of sympathetic nervous system (SNS). It can result in lasting alterations in autonomic, neurohormonal, brain, and peripheral function, and in extreme cases symptoms of PTSD [44]. This chapter provides physiology background for stress, PTSD, and vagus nerve that are tied to the findings and methods in the next chapters.**Neurobiology of Stress and Stress-Related Disorders**

The neurobiology of stress is complex and involves interconnected molecular pathways of the sympathetic nervous system, the hypothalamic pituitary adrenal (HPA) axis, inflammatory response systems, and other neuropeptidal and neurohormonal systems [44, 45]. By the introduction of stressful input, a cascade of events take place starting from the neural circuitry to downstream events such as motor, inflammatory, hormonal, and physiologic responses in a physically healthy individual, as seen Figure 2, adapted from [46]. The thalamus acts as the gateway to the brain, filtering sensory input from the outside world. A network of hippocampus, amygdala, and prefrontal cortex are hypothesized to function in neural circuitry of fear. The hippocampus (that has a major role in memory and learning) has connections with amygdala, which plays role in processing emotions and fear. The medial prefrontal cortex is hypothesized to play a role in the extinction of fear responses. The operation of this network during sensory input acquisition leads to motor, hormonal, and physiological responses. For patients with PTSD, this brain network is

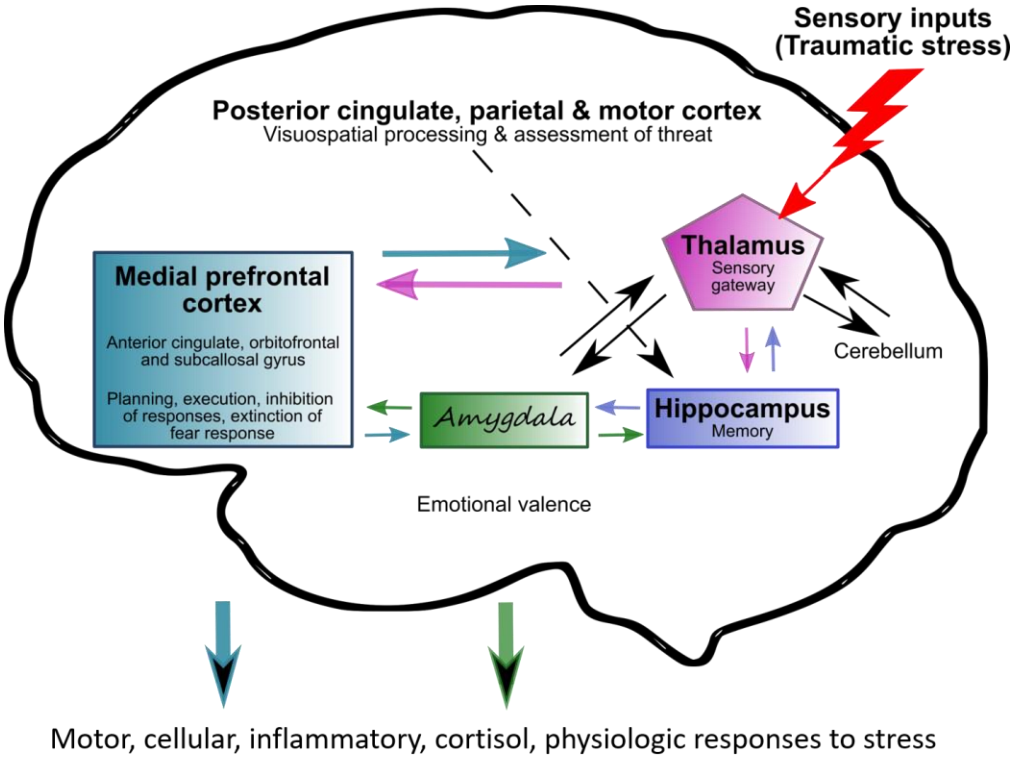

Figure 2-1. Neural circuitry of stress. Adapted from [46].

dysfunctional: Patients with PTSD have lower hippocampal volume. Medial prefrontal cortex, that takes part in the inhibition of fear response, is dysfunctional. This results in a failure of extinction in fear response. As a result, they exhibit abnormal/blunted motor, hormonal, and physiologic responses. Exposure to events or stress—particularly those with salient characteristics related to previously experienced trauma—can elicit symptoms such as hyperarousal, intrusive thoughts, avoidance behaviors, and dissociation, and dysfunctionalizes this cascade of stress processing [47]. These adverse responses can lead to elevated inflammatory marker concentrations, impaired autonomic modulation, memory deficits, changes in brain morphology, and increased neural reactivity in emotion-specific brain areas [48-56]. Laboratory-produced stress paradigms are known to produce an acute stress response and increase SNS activity in previously traumatized individuals with and

without PTSD [51, 57, 58]. Exposure to stress activates neural areas repeatedly found to be altered with PTSD and related psychiatric disorders [51, 59]: the amygdala, prefrontal cortex, anterior cingulate, hippocampus, and insula. A summary is provided below addressing the physiologic, immune, and cellular responses in stress and PTSD.

2.1.1 Dysregulated Immune Function in Stress and PTSD

Dysregulated immune function is associated with stress and PTSD [60, 61]. Mental stress in the laboratory in human subjects, including patients with coronary artery disease [62], is associated with increases in several inflammatory markers, including interleukin-6 (IL-6) [63-65], IL-1ß [65], IL-10 [65], and tumor necrosis factor-alpha (TNF-α) [65]. Multiple studies show an increase in inflammatory factors at baseline in patients with depression [66-70] and early trauma [71-75]. Consistent with these studies, PTSD patients have increased inflammation [56], including increased baseline concentrations of leukocytes [76-78], IL-6 [60, 79-87], IL1ß [60, 88, 89], TNF-α [60, 80, 83, 86-89], Interferon (IFN)-γ [60, 85, 89, 90], intercellular adhesion molecule-1 (ICAM-1) [91, 92], vascular cell adhesion molecule-1 (VCAM-1) [92], and in one study, IL-2, IL-4, IL-8, and IL-10 [85]. Other studies showed no increase in IL-6 [88, 91, 93-95], CRP [87, 89, 92, 93, 95, 96], IL-4 [88], IL-10 [80, 88, 89], IL-1ß [89], or IFN-γ [93]. One study found increased diurnal cerebrospinal fluid (CSF) IL-6 but not plasma IL-6 in PTSD [97]. Other studies showed altered genotype in genes modulating immune function in PTSD [98]. Recently, enhanced IL-6 response to mental stress involving public speaking in coronary artery disease [62] patients with PTSD compared to CAD patients without PTSD [99]. In summary, studies implicate altered immune function in PTSD including increased IL-6, TNF-α and IFN-γ.

2.1.2 Enhanced Cellular Stress Response in PTSD

Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide that connects these systems and regulates and integrates adaptive responses to stress [100]. A growing body of literature has pointed to dysregulation of PACAP along with its selective PAC1 receptor in PTSD [100, 101]: Elevated PACAP levels predicted symptom clusters categorized as intrusion, avoidance, and hyperarousal in females with PTSD. In other studies, PAC1 receptor levels were also correlated with physiological measures (increased startle reflex, related to the hyperarousal category of PTSD diagnosis) that have been previously associated with PTSD risk [102-108]. Experiments demonstrated that PACAP is related to physiological stress responses including those mediated by the sympathetic and parasympathetic nervous system [109]. PACAP is released along with acetylcholine from nerve terminals in the adrenal gland, and it targets cells that express the nicotinic receptor along with PACAP receptors [109]. Accordingly, evidence suggests a functional adrenomedullary synapse in which acetylcholine and PACAP act independently to cause release of catecholamine [109].

Within the brain, PACAP participates in neural circuits relevant to PTSD and other stress disorders [110]. In the hypothalamus and hippocampus, PACAP acts as an important neuromodulator [111, 112]. Anatomical and physiological studies established the importance of PACAP neurotransmission in the amygdala, which underscored the role of this peptide transmitter in fear responses and potentially PTSD [109, 113]. In summary, studies suggest that PACAP is anxiogenic and may play a role in symptoms of PTSD.

2.2 Vagus Nerve Stimulation

Treatments for PTSD hinge on the mitigation of stress reactivity, including reducing brain activity, inflammation, cellular, and physiological responses to stress. Neuromodulation, which involves modulating neural state based on external inputs to the body that typically are electrical, acoustic, or magnetic, may provide a novel means of mitigating stress reactivity if appropriate nerve targets and modulation paradigms are discovered. The vagus nerve might be an appropriate target for neuromodulatory therapies in patients with PTSD due to its afferent connections to key regions in the brain involved in stress neurobiology, and to its efferent signaling to peripheral organs such as the heart.

The vagus nerve, the longest cranial nerve, is a complex neural structure that contains descending efferent fibers that regulate peripheral organs and autonomic nervous system activity, and ascending afferent fibers to the brain via the nucleus tractus solitarus (NTS) [114]. The NTS projects to other brain areas such as the amygdala, hippocampus, locus coeruleus, and prefrontal cortex that play important roles in emotion regulation and have been implicated in stress-related mental disorders, including PTSD [115]. Efferent fibers modulate cardiovascular function and peripheral autonomic tone, which can also be modulated by afferent fibers via brain areas with effects on these parameters including the prefrontal cortex and insula [116]. Electrical stimulation of the vagus nerve, using implantable devices (direct VNS), has been demonstrated to be efficacious for the treatment of epilepsy and refractory major depression, and is approved by the Food and Drug Administration (FDA) for the treatment of these disorders [117-121]. The effects of direct VNS on autonomic imbalance likely explains much of its efficacy for these disorders, as well as its applicability to cardiovascular disorders [122, 123]. The effects of

direct VNS on enhancement of memory and neuroplasticity also suggest a role for treatment of cognitive disorders, stroke, and other conditions [124-128].

2.2.1 Relevance of Vagus Nerve Stimulation and Neurobiology of Stress

VNS has effects on inflammation that may be beneficial for PTSD. IL-6 and TNF-α are modulable by the vagus nerve [129-134]. In animal studies VNS blocks lipopolysaccharide (LPS)-induced increases in IL-6, IL-18, IL-1ß and TNF-α, but not IL-10 [131, 135]. Studies in patients with epilepsy and implanted VNS devices showed that long-term treatment resulted in decreased LPS-induced IL-6 and neurotoxic kynurenic metabolites with no effect on IL-6, IL-10, IL-1ß, or TNF-α [136, 137].

Besides the relevance of vagus nerve and immune function, cellular stress neuropeptide PACAP also has neuroprotective and anti-inflammatory properties and is well-positioned for modulation by vagus nerve activity [138, 139]. Of particular interest to the vagus nerve, PACAP innervation of the lateral central amygdala is thought to arise from PACAP containing neurons in the vagus nerve brainstem complex [113]. Behavioral studies in rodents indicate that PACAP exerts an anxiogenic effect via its connections in the bed nucleus of the stria terminalis (BNST, also referred as extended amygdala) [140]. A growing literature on the anatomical location and physiology of PACAP suggested a close association with systems that are also regulated by VNS [141-143]. Therefore, PACAP is well-positioned as a mediator in pathways of the cholinergic anti-inflammatory axis and its potential modulation by VNS. PACAP may potentially serve as a dynamic and objective biochemical biomarker that could measure PTSD severity, hence the longitudinal changes in PACAP may indicate therapy response to potential treatments targeted at PTSD. The

relationship between PACAP levels, stressful stimuli, and VNS (either direct or noninvasive stimulation) has not been examined in humans before.

In summary, the HPA axis plays an important role in stress, and dysregulation of this system is associated with PTSD and depression, and is potentially modifiable by the vagus nerve. FDA-approved direct VNS involves surgical implantation with direct electrical stimulation of the vagus nerve. VNS has effects that may be beneficial for neurophysiological alterations associated with PTSD, including blocking of sympathetic and immune function [144-146], and enhancement of cognition [124, 147-151]. The requirement for surgical implantation, however, has limited the widespread implementation of VNS to psychiatry due to cost, inconvenience [152, 153], and lack of reimbursement by Medicare or other insurance companies [154].

2.2.2 Non-invasive Vagus Nerve Stimulation: Current State of the Art

Due to the limitations of surgical implants, noninvasive vagus nerve stimulation (nVNS) devices have been developed that target the projections of the vagus nerve from the skin. Transcutaneous VNS devices noninvasively target vagal projections in the ear (auricular branch of the vagus) and neck (cervical branch in the carotid sheath). Auricular tVNS (taVNS) devices modulate central and peripheral physiology, as observed with the monitoring of peripheral physiological parameters [155-157], inflammatory cytokines [158], hormonal indices [159], brain imaging [160-163], and a case study comparing VNS implant and taVNS for an epilepsy patient [164]. taVNS also has been shown to ameliorate tinnitus [165, 166], atrial fibrillation [167], episodic migraine [162], seizure frequency [168], cluster headache [169], and major depression [170, 171], as well as improving vagal

tone [157], deactivation of limbic and temporal brain structures, and mood enhancement in healthy populations and patients with PTSD and mild traumatic brain injury [172, 173]. Fewer studies have looked at cervical tVNS (tcVNS), although they have been shown to reliably activate vagal nerve fibers [174-176], produce anti-inflammatory effects [177-179], reduce neural and physiologic responses to noxious thermal stimuli [180] with possible clinical utility in migraine and trigeminal allodyna [181, 182] . The work in this dissertation focuses on tcVNS paired with traumatic recall and mental stress tasks in individuals with or without PTSD.

2.3 PTSD Diagnosis: Current State of the Art

Diagnosis of PTSD requires exposure to at least one event that involved threat of death, violence, or injury. An individual could be exposed in one or more ways: direct experience, witnessing, learning someone close to the individual faced the event, or repeated exposure to traumatic event cues (such as police officers repeatedly exposed to details of child abuse). According to DSM-5 [39], the following symptoms are required to be diagnosed as PTSD, existence and degree determined after a structured interview:

- Criterion B: Intrusion symptoms (one required)
    - The traumatic event is persistently re-experienced in the following way(s):
        - Unwanted upsetting memories
        - Nightmares
        - Flashbacks
        - Emotional distress after exposure to traumatic reminders
        - Physical reactivity after exposure to traumatic reminders

- Criterion C: Avoidance (one required)
    - Avoidance of trauma-related stimuli after the trauma, in the following way(s):
        - Trauma-related thoughts of feelings
        - Trauma-related external reminders
- Criterion D: Negative alterations in cognitions and mood (two required)
    - Negative thoughts or feelings that began or worsened after the trauma, in the following way(s):
        - Inability to recall key features of the trauma
        - Overly negative thoughts and assumptions about oneself or the world
        - Exaggerated blame of self or others for causing the trauma
        - Negative affect
        - Decreased interest in activities
        - Feeling isolated
        - Difficulty experiencing positive affect
- Criterion E: Alterations in arousal and reactivity
    - Trauma-related arousal and reactivity that began or worsened after the trauma, in the following way(s):
        - Irritability or aggression
        - Risky or destructive behavior
        - Hypervigilance
        - Heightened startle reaction

        - Difficulty concentrating
        - Difficulty sleeping
- Criterion F: Duration (required)
    - Symptoms last for more than 1 month.
- Criterion G: Functional significance (required)
    - Symptoms create distress or functional impairment (e.g., social, occupational).
- Criterion H: Exclusion (required)
    - Symptoms are not due to medication, substance use, or other illness.

In summary, the current state of the art for diagnosing PTSD requires interaction with a medical professional and an approximately one hour long structured interview. In addition, the interviews rely on the individual's reporting, thus susceptible to malingering or under-reporting the specific categories [183, 184]. An objective assessment could potentially help in the diagnosis of PTSD. This objective assessment could potentially be achieved through noninvasive quantification of the autonomic reactivity to a stressful stimuli.

This chapter introduced neurobiology of stress and stress-related disorders, and the potential of noninvasive vagus nerve stimulation to mitigate stress. Stress-related disorders lead to systemic inflammation, enhanced cellular stress response, and autonomic imbalance. The neurobiological and physiological targets reviewed in this chapter laid out the rationale for investigating noninvasive vagus nerve stimulation in stress and PTSD.

CHAPTER 3. QUANTIFYING STRESS REACTIVITY

3.1 Introduction

The autonomic nervous system (ANS) is a division of the peripheral nervous system that influences the function of internal organs. It acts largely unconsciously and regulates bodily functions. Traditional literature suggests that ANS is dominated by two main branches: sympathetic (fight-or-flight, SNS) and parasympathetic (rest-and-digest, PNS) branches, while polyvagal theories exist that do not limit the dominant branch number to two [185]. In many cases, these branches have opposite actions where one system activates a physiological response and the other inhibits it, while other cases such as vasovagal syncope (i.e., fainting) are also possible [186]. ANS activity plays a critical role in stress response, and autonomic imbalance plays an important role in the progression of many cardiovascular and nervous system disorders [187-189]. This chapter introduces two works related to autonomic nervous system activity quantification in the context of stress [190, 191]. These works underscore the importance of multimodal autonomic monitoring.

3.2 Noninvasive Biomarkers of Autonomic Nervous System Activity

As the ANS affects internal organ functions, noninvasive monitoring of internal organ functions is critical in quantifying the ANS activity. These physiological data could be collected with off-the-shelf or custom-made sensors. The physiological measurements include but not limited to electrocardiography (electrical activity of the heart, ECG), respiration (respiratory effort, RSP), seismocardiography (chest wall vibrations of heart, SCG), photoplethysmography (blood volume pulse, PPG), electrodermal activity (sweat

gland activity, EDA), blood pressure (BP), impedance cardiography (thorax bioimpedance modulated by blood ejected from the heart, ICG), and near infrared spectroscopy (relative prefrontal cortex oxygenation, NIRS).

3.2.1 Heart Rate

The instantaneous heart rate (HR), computed from R-R intervals of ECG is a traditional measure of stress response. HR is controlled both by SNS and PNS activity. The net effect of the ANS on HR is often seen as the net balance between two branches [192]. The interplay between the two and additional effects such as baroreflex or chemoreflex sensitivity might mask the true sympathetic activation. Regardless, it is a convenient measure to obtain from different sensors (ECG, PPG, SCG, ICG) providing heartbeats [193].

3.2.2 Heart Rate Variability

Heart rate variability (HRV) is a general name for methods that quantify the variation in time intervals between heartbeats. There are a number of frequency and time domain HRV measures, popular in clinical literature. HRV measures tend to be non-continuous (not beat by beat) as multiple clean beats are required to compute the power of specific frequency bands or quantify beat variation [194]. Frequency-domain HRV, the most commonly studied method for quantifying the sympathetic and parasympathetic branches of the ANS, is obtained from the non-constant R-R intervals from ECG R-peaks [195]. While the power in the high-frequency range (HF HRV, 0.15-0.4Hz) is considered a measure of parasympathetic activity for humans, the low-frequency portion (LF HRV, 0.04-0.15Hz) is mostly used for assessing the changes related to both sympathetic and parasympathetic

influences [195]. The ratio of the two power bands (LF/HF) is often considered as a measure of sympathetic tone, while there are discrepancies in the literature [196, 197].

Poincaré method is a less commonly employed HRV measure, obtained from the scatter plot of each R-R interval (R-R_n) versus the next R-R interval (R-R_{n+1}). In this procedure, an ellipse is fitted to the line-of-identity of the scatter plot (R-R_n versus R-R_{n+1}). Three indices are established from the fitted ellipse: standard deviation (SD) of points perpendicular to the axis of line-of-identity (SD1), standard deviation of points along the axis of line-of-identity (SD2), and their ratio (SD1/SD2). SD1 measures short-term HRV which correlates with baroreflex sensitivity (BRS, change in the inter-beat interval duration per unit change in BP) and HF HRV. SD2 measures short- and long-term HRV and correlates with BRS and LF HRV. The ratio SD1/SD2 (the unpredictability of R-R intervals) is an indicator of the autonomic balance [198, 199].

3.2.3 Amplitude of Peripheral Photoplethysmogram

The amplitude of the peripheral photoplethysmogram (PPG) signal is known to be affected by sympathetic and vasomotor activity [200], and environmental effects such as temperature [201]. In our studies described in the current and next chapters, we observed that it typically decreases with mental stress perturbations on the autonomic state.

3.2.4 Pre-Ejection Period of the Heart

The pre-ejection period, measured by the time delay between the onset of electrical depolarization (QRS waveform, obtained from ECG) and aortic valve opening, is considered as a non-invasive indicator of cardiac SNS activity [202, 203]. Specifically,

decreased PEP is a surrogate measure of increased cardiac contractility, which may change acutely due to autonomic influences. In particular, decreased PEP reflects increased cardiac β1 receptor stimulation [202]. PEP is an underused measure due to the requirement for a second modality in addition to ECG: ICG or SCG to capture the electromechanical activity of the heart. A traditional way to measure PEP is through ECG and ICG or ECG and heart sounds, and ICG alone requires at least four electrodes on body. Instead of ICG, SCG or ballistocardiography (BCG), by giving information on the electromechanical coupling of the heart, could provide high quality PEP estimation when combined with ECG [204, 205].

3.2.5 Pulse Transit and Arrival Times

Pulse transit time (PTT) represents the time it takes for the pressure wave to travel from a proximal to a distal location along the arterial tree, traditionally measured by two BP waveforms [200]. PTT is inversely related to continuous BP through Moens-Korteweg equation (Equation 3.1), assuming a constant distance travelled for the arterial tree:

$$BP = \frac{K_1}{PTT} + K_2 \tag{3.1}$$

Where K_1 and K_2 are subject-specific parameters that take into account the length of measurement site, arterial diameter, and arterial elasticity [206]. As such, changes in PTT are related to changes in continuous blood pressure.

$$PAT = PEP + PTT \tag{3.2}$$

A common though inaccurate alternative to PTT is the measurement of pulse arrival time (PAT), the timing interval between the R-wave of ECG and the pulse wave at a distal

location. PAT is the sum of PEP and PTT (Equation 3.2). Compared to only PTT calculation, PAT calculation has advantages in terms of simplicity and ease of use, however PAT contains PEP in addition to PTT. PEP could change independently from BP, due to changes in cardiac contractility [207, 208]. It should be noted that as PAT includes both sympathetic influences due to PEP and vasomotor influences due to BP, it will provide a higher net change due to autonomic influences driving the sympathetic and vasomotor activity to the same direction.

3.2.6 Respiratory Measurements

Slowly varying respiratory activity is modulated by PNS activity [195]. Respiration frequencies are around 0.4Hz for humans, respiration activity could be extracted from other signals (ECG, SCG, PPG, ICG) with proper low-pass filtering or by the use of a piezoresistive belt that captures thoracic expansion and contraction while breathing. The characteristics of the respiration cycle (rate or RR, width or RW, prominence or RP) are of interest in terms of PNS activity and breathing pattern.

3.2.7 Electrodermal Activity

The EDA signal is a measure of sweat gland activity, and composed of two main components. The slow tonic component (skin conductance level, SCL) shows the general trend of the signal. The faster tonic component (skin conductance response, SCR) is superimposed onto the tonic component. EDA is typically taken from palms where large number of sweat glands exist [209].

3.2.8 Prefrontal Cortex Oxygenation Measurements

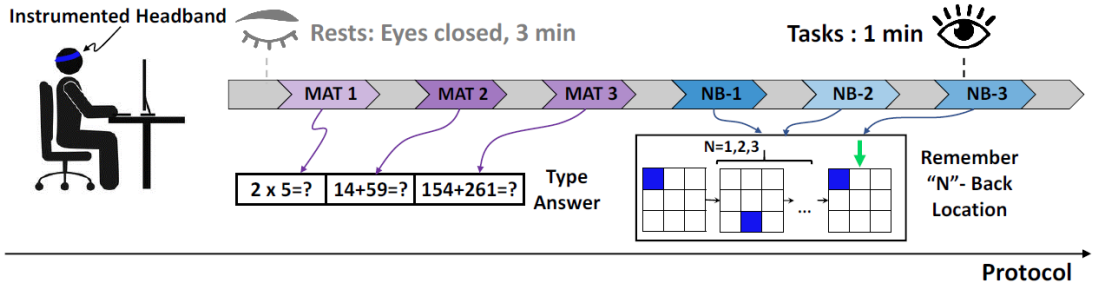

Figure 3-1. Protocol description: Each subject completed a series of arithmetic and N-back tasks with difficulty levels shuffled, each lasting a minute. Before the start of the tasks and between each task, subjects rested for three minutes with their eyes-closed. MAT: Mental arithmetic, NB: N-back task.

NIRS is an optical and non-invasive method for monitoring the changes in tissue oxygen dynamics. NIRS provides information regarding blood flow to the pre-frontal cortex (PFC) by use of the Modified Beer-Lambert Law (MBLL) (Equation 3.3), which allows calculation of oxygenation changes from light intensity measurements scattered from human forehead, or any other body area [210]. When a tissue is activated due to autonomic perturbations, it uses oxygen. Oxygen transport is satisfied by hemoglobin. Hemoglobin has useful absorption spectra in near infrared (NIR) spectrum, which can be leveraged to monitor relative concentration changes.

$$\begin{bmatrix} \Delta A_{\lambda_1} \\ \Delta A_{\lambda_2} \end{bmatrix} = \begin{bmatrix} \varepsilon_{\lambda_1}^{HbR} d & \varepsilon_{\lambda_1}^{HbO} d \\ \varepsilon_{\lambda_2}^{HbR} d & \varepsilon_{\lambda_2}^{HbO} d \end{bmatrix} \begin{bmatrix} \Delta c^{HbR} \\ \Delta c^{HbO} \end{bmatrix} \tag{3.3}$$

Where $\lambda_{1,2}$ are measurement wavelengths, A is absorbance, c is concentration, HbO is oxyhemoglobin, HbR is deoxyhemoglobin, $\varepsilon_{\lambda_1}^{HbO}$, $\varepsilon_{\lambda_1}^{HbR}$, $\varepsilon_{\lambda_2}^{HbR}$, $\varepsilon_{\lambda_2}^{HbO}$ are extinction coefficients at $\lambda_{1,2}$ of HbO and HbR (typically approximated constant), and d is distance between light source (NIR, 770-850nm) and detector (matched photodiode). By measuring

A (ratio of input and output light intensity) at $\lambda_{1,2}$ and plugging in $\varepsilon_{\lambda_1}^{HbO}$, $\varepsilon_{\lambda_1}^{HbR}$, $\varepsilon_{\lambda_2}^{HbR}$, $\varepsilon_{\lambda_2}^{HbO}$, d, one could solve for concentration changes Δc^{HbR} and Δc^{HbO}.

3.3 Fusing Near-Infrared Spectroscopy with Wearable Hemodynamic Measurements Improves Classification of Mental Stress

This work established an instrumented headband to classify mentally stressful tasks using various biomarkers of ANS activity and PFC oxygenation [191]. NIRS is typically used in conjunction with electroencephalography [211] [212, 213] with bulky setups to improve spatial and temporal information, this work combined central (NIRS) and local (cardiovascular and peripheral) responses with an unobtrusive setup with the goal of classifying the mentally stressful state.

3.3.1 Human Subject Study Design

The human subject study was performed under a protocol reviewed and approved by the Georgia Institute of Technology Institutional Review Board. All subjects read and signed

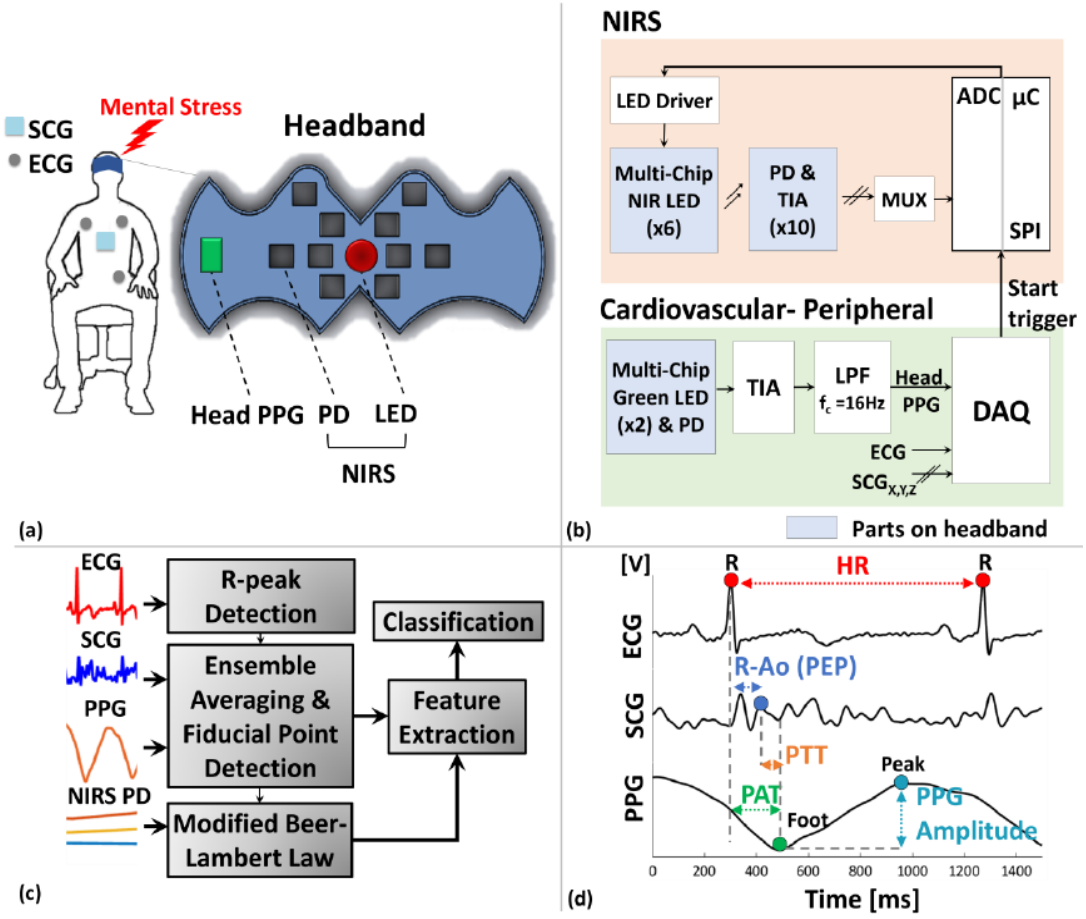

Figure 3-2. a) Illustration of the sensing modalities attached to each subject: headband PPG, NIRS, ECG, SCG signals were collected throughout the mental stress protocol. b) Block-diagrams of each sensing modality. c) Block diagram of signal processing and feature extraction. d) Summary of peripherally-measured cardiovascular parameters. HR, R-Ao or PEP, PTT, PAT, PPG amplitude were extracted from the peripherally measured cardiovascular sensing part.

a consent form before the data collection. Data were collected from 16 healthy subjects without cardiovascular disorders (six females, ten males, ages 26.7 ± 3.2, mean ± SD). Figure 3.1 illustrates the experimental protocol and Figure 3.2a shows the test setup for each subject. The experiment was divided into six parts that include mental tasks (mental arithmetic and N-back), with three-minute breaks in between each task. The arithmetic task was chosen due to the high cardiovascular response observed in many clinical studies

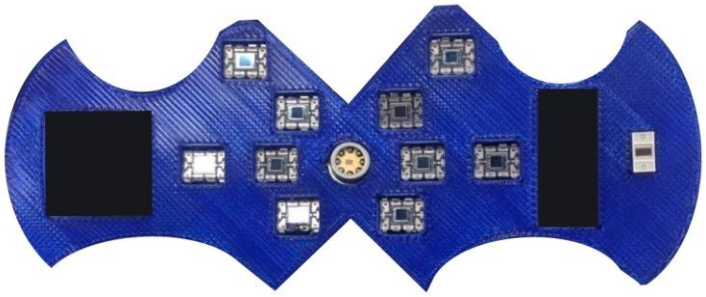

Figure 3-3. Instrumented headband.

relative to other mental stress tests [214]. The N-back task was chosen because of its relevance with PFC activity that could be captured with NIRS [215-217]. All tasks were carried out on a laptop and the subjects used a keyboard to interact with custom graphical user interfaces (GUIs). The subjects were asked to remain silent, and to minimize posture changes during the protocol. Before the start of the experiment, the protocol was explained in detail to each subject, and the subjects practiced sample questions from each task. At the beginning of the experiment, each subject was instructed to sit comfortably with eyes closed for three minutes to obtain baseline rest signals. Then, the subjects underwent a series of arithmetic and N-back tasks, with difficulty levels ordered randomly. The questions in these tasks were different from the ones in the practice session. The three arithmetic tasks included 1-digit, 2-digit, or 3-digit algebraic calculations from a custom GUI. A series of arithmetic questions appeared on the screen for each difficulty level, for one-minute each, as illustrated in Figure 3.1. The subjects entered the answers using the keyboard and pressed a key to progress onto the next question. The subjects were not allowed to progress to the next question if they did not enter the right answer. After completing the first three tasks that include arithmetic questions, subjects progressed to the N-back task. The N-back task is a continuous performance task to measure working

memory and working memory capacity. In this task, a three-by-three grid was presented to the subject on the computer screen via a GUI [19]. A sequence of squares at different spatial locations were highlighted consecutively, and the subject pressed a key when the location of the current highlighted square matched the one from N-steps earlier in the sequence (Figure 3.1). Our protocol included N = 1, 2, 3-back tasks shuffled randomly, each lasting for a minute. Each trial (square appearance) was adjusted to last for a maximum of three seconds, therefore each N-back task includes approximately 20 trials within a minute.

To assign the subjective difficulty levels for the classification of each task, the subjects filled out the NASA Task Load Index (NASA-TLX) questionnaire at the end of the protocol [218]. For each of the six tasks (three arithmetic and three N-back), the total workload was divided into six subcategories: mental demand, physical demand, temporal demand, performance, effort, frustration. The subjects rated each subscale with a score between 0-100, for each task. The ratings from the six subcategories were averaged for each task, referred to as RTLX scores. The average score indicates an estimate of overall workload for the corresponding task [218]. The use of RTLX objectifies the difficulty assignment for each task type, verifying that the protocol was effective. The highest difficulty levels were used for the classification.

3.3.2 Instrumentation

Figure 3.2a and b show the parts of headband and instrumentation blocks, namely NIRS and cardiovascular-peripheral blocks. The NIRS circuit consisted of a multi-chip NIR light emitting diode (LED) (MTMD7885T38, Marktech Optoelectronics, Latham, NY), 10 photodiode-transimpedance amplifier (PD-TIA) chips (OPT101, Texas Instruments,

Dallas, TX), 16-channel LED driver (TLC5940, Texas Instruments, Dallas, TX), 16-channel multiplexer (MUX, CD74HC4067, Texas Instruments, Dallas, TX), and a microcontroller (μC, ATmega 2560, Arduino, NY). The multi-chip LED included six LEDs, with peak emission wavelengths (λ) of 770nm (x 2), 810nm (x 2), and 850nm (x 2). The package had a diameter of 9mm, and all LEDs were separated by 45°. To maximize the level of reflections received by the detector, the LED driver was programmed such that the LEDs would operate with the maximum forward currents recommended by the manufacturer: 50mA for 770nm and 100mA for 810nm and 850nm. The MUX output was programmed to sequentially select which detector output would be read by the built-in analog-to-digital converter (ADC, 10-bit) of the microcontroller (time division multiplexing). A flexible headband was designed in SolidWorks (2016, Waltham, MA), as shown in Figure 3.3, with dimensions 140.5mm by 58.5mm by 4.5mm, using thermoplastic polyurethane (TPU) filament (NinjaFlex, Manheim, PA). The NIRS LED was placed to the center of the headband, and 10 of PD-TIA chips were distributed spatially around the NIRS LED. The distances between each source (LED)-detector (PD-TIA) combination were set at 1.5cm or 3cm. The distances were chosen due to the known optimal sensitivity of NIRS to intracranial brain tissues at these distances, and the NIRS implementations in literature [219].

In terms of firmware, upon the detection of a trigger signal from a data acquisition system (DAQ, MP150, Biopac Systems, Goleta, CA), the NIRS LEDs were programmed to turn on sequentially. Once an LED was turned on, the MUX sequentially selected detectors to read their output signals. The NIRS signals were transmitted with 2Hz sampling rate. For the peripherally measured cardiovascular signals, both custom-built circuits and

commercially available components were used to acquire a set of signals that would capture at least the following physiological features of relevance for mental stress assessment: HR, PEP, PAT, PTT, and PPG amplitude. The goal was to use sensors and electronics for measuring these signals that could ultimately be encapsulated in a wearable device, possibly even a headband with combined NIRS and cardiovascular signal sensing capability.

The headband contains the head PPG sensors, which include a multi-chip LED and PD combination (SFH 7070, OSRAM Optosemiconductors, Regensburg, Germany). The package includes two green emitters (λ = 530nm) and a matched PD, with an overall dimension of 7.5mm by 4mm by 0.9mm. The forward current through the LEDs were set to 20mA, per the datasheet suggestion, using a voltage divider and buffer combination. A TIA was designed to read the PD output using an operational amplifier (LT1885, Linear Technology, Milpitas, CA) with feedback components (R_F = 350kΩ, CF = 10nF), followed by a first-order passive low-pass filter (f_c = 16Hz). The head PPG signal was acquired using the DAQ. To measure SCG signals, a very low-noise 3-axis accelerometer evaluation board was used (ADXL354CZ, Analog Devices, Norwood, MA). The accelerometer was placed in a 2.8cm by 3cm by 1cm custom-printed rigid acrylonitrile butadiene styrene plastic case. It was placed on the mid-sternum of each subject. For ECG data collection, a commercially available wireless 3-lead ECG amplifier was used (RSPEC-R, Biopac Systems, Goleta, CA). All peripherally measured cardiovascular signals were transmitted through the DAQ with 2kHz sampling rate.

All custom circuits were powered using a benchtop power supply with ±9V rails. For the components that require specific power levels (i.e. 3.3V for accelerometer and 5V for LED

driver and PD-TIA), low drop-out regulators were used (LT1763, Linear Technology, Milpitas, CA).

3.3.3 Signal Processing and Feature Extraction

Pre-Processing and Feature Extraction: Data were processed in MATLAB (R2017b, MathWorks, Natick, MA). Figure 3.2c gives an overview of the signal processing and feature extraction pipeline. The peripherally measured cardiovascular parameters extracted are cardiac timing intervals and a signal amplitude, namely: the HR, R-Ao (i.e., PEP), PAT, PTT, and head PPG amplitude. NIRS parameters are changes in concentrations of oxy-hemoglobin (ΔHbO), deoxy-hemoglobin (ΔHbR), and total hemoglobin ($\Delta TotalHb$). Figure 3.2d shows the parameters computed from the peripherally measured cardiovascular signals. ECG, SCG and head PPG signals were filtered with finite impulse response (FIR) band-pass filters with cut-off frequencies 0.6-40 Hz, 0.8-20 Hz, and 0.8-10 Hz, respectively, to preserve the waveform shape and cancel the noise outside their bandwidths [220]. The R-peaks of the ECG signals were detected using thresholding, and were used to calculate HR. SCG and head PPG signals were ensemble averaged according to the R-peaks, using beat lengths of 300ms for SCG and 550ms for the head PPG. These lengths were sufficient to detect the fiducial points of each SCG and PPG beats. To reduce the effects of motion artifacts on the individually segmented beats, exponentially weighted moving ensemble averaging of successive beats was implemented [220]. Exponentially decreasing weighting gives more emphasis to the more recent beats, while still providing noise reduction based on the averaging. We determined 3-beat and 10-beat time constants for SCG and head PPG were sufficiently long to reduce the artifacts while short enough to still preserve the transient changes in the signals. Note that for all the parameters described

below, the approximate first order derivatives (differences between the adjacent elements) were also computed to generate additional parameters.

PEP: The time interval between the R-peak of the ECG to the second peak of the SCG (known as aortic opening point, AO) is known to be highly correlated with PEP [204]. Therefore, we used R-Ao as a measure of sympathetic activity.

PAT, PTT, PPG amplitude: PAT represents the time delay from the electrical depolarization of the ventricles to the arrival of the pulse to forehead region, where the PPG signal is collected. We also calculated PTT as the time interval between the AO point of the SCG signal to the foot of the head PPG signal. As a measure of peripheral sympathetic and vasomotor activity, the amplitude of PPG signal was extracted.

PFC Oxygenation Markers: The changes in oxy-hemoglobin, deoxy-hemoglobin, and total hemoglobin concentrations (ΔHbO, ΔHbR, $\Delta Total\ Hb$) were calculated from NIRS signals according to MBLL [210]. The NIRS channel to process with MBLL was chosen manually due to interference from hair on forehead and incomplete contact of a few detectors for some subjects.

Normalization and Dataset for Classification: After the difficulty level assignment for each task using RTLX, the tasks that resulted in the maximum perceived workload were selected to be used in the classification. Specifically, the parameters used for classification were extracted from rest (one minute), hard arithmetic (one minute), and hard N-back (one minute). Then, peripherally measured cardiovascular parameters were normalized using a baseline reference interval. The one-minute reference interval for peripherally measured cardiovascular parameters was collected before the protocol started. This interval is

different than the rest class interval. NIRS parameters were used as is as MBLL already implements normalization. To equalize the length of each parameter within an interval, extracted parameters were resampled to the length of the parameter that has the maximum length. Then instances were created by using 10-sample sliding windows with 50% overlap. The features used in the classification consisted of the mean, standard deviation (std), maximum (max), minimum (min), area under curve (auc) and slope of the extracted parameters in each window. There was a total of 88 features (33 NIRS features, 55 peripherally measured cardiovascular features), and the total number of instances were 842 (333 rest instances, 228 arithmetic instances, 281 N-back instances).

3.3.4 Machine Learning

For the classification of mental tasks and rest state, a feature matrix was constructed from all extracted features and the corresponding labels as classes (rest, arithmetic, N-back classes). This matrix included instances as rows and features as columns, and it was used to build the classification model. To eliminate irrelevant features that could decrease the accuracy of the classification model, we performed feature selection. Univariate feature selection was performed by calculating p-values for each feature using analysis of variance (ANOVA), and applying the Benjamini-Hochberg procedure ($\alpha = 0.005$) for multiple comparisons [221]. A univariate statistics-based feature selection method rather than manual selection automates the feature selection process, making it possible to treat the data blindly without assumptions. To visualize the dataset, we performed the aforementioned feature selection on the whole dataset and applied t-distributed stochastic neighbor embedding (t-SNE), reducing the dimensionality of the dataset to two [222].

To classify each instance to one of three mental tasks using the selected features, a Random Forest classifier was used. A random forest classifier is an ensemble learning method that trains multiple decision trees and determines the classification result through a majority vote amongst all individual trees. Each tree is trained on a bootstrap sample drawn from the dataset, and at each node of the tree, a random subset of the features is considered for a split [223]. In our algorithm, we trained 50 trees as a part of the random forest classifier. A single hyperparameter of the trees, maximum depth, was tuned using a leave-one-subject-out cross validation (LOSO-CV) grid search scheme. In this scheme, we first defined the parameter grid values between three to ten. For each value on the grid, we performed LOSO-CV and found the parameter that maximizes the LOSO-CV accuracy, to use that parameter in the final model. The maximum depth parameter controls the complexity of each tree in the forest where increased depth corresponds to more complicated models.

Random forests are ensemble learning models that are often hard to interpret, especially when they consist of many trees. To get more insight on what the model learned, we performed feature importance ranking using a random forest classifier that was trained on the whole dataset. This was done by evaluating the improvement in the gini-index metric at each node of each tree within the forest. These improvements were accumulated across all nodes of all trees within the forest to rank the feature importance, with the most important features resulting in the largest improvements in the gini-index [223]. Feature selection, classification and t-SNE dimensionality reduction were all implemented using the scikit-learn library for Python [224].

3.3.5 Model Evaluation

We evaluated our algorithm using LOSO-CV. Without this subject's data, we first performed feature selection followed by hyperparameter tuning via grid-search within an inner LOSO-CV loop. With the optimal hyperparameters and the selected features, we trained our random forest classifier which was then used to calculate the performance metrics: accuracy, precision, recall and F1 score for each subject. The final scores were calculated by averaging the scores from each CV fold.

It should be noted that each CV fold implements an inner CV loop on the subjects that are not left out, which results in a nested CV protocol [225]. This procedure was performed by using only NIRS features, only peripherally measured cardiovascular features and the fusion of both sensing modalities. The results were compared using statistical analyses to identify which sensing modalities perform better in differentiating among the rest, arithmetic, N-back tasks.

3.3.6 Statistical Analysis

We performed statistical analyses on the classification results to compare each sensing modality alone and their fusion. Specifically, each LOSO-CV fold results in one data point (accuracy, precision, recall, or F1 score metrics) per subject. We obtained 16 data points for 16 subjects per metric. This scheme was repeated for NIRS alone, cardiovascular (cardio) alone, and the fusion of both. These samples were used for statistical testing to assess the performance of the sensing modalities. Friedman Test was chosen to detect if any difference exists between the performance of the sensing modalities from the outcomes

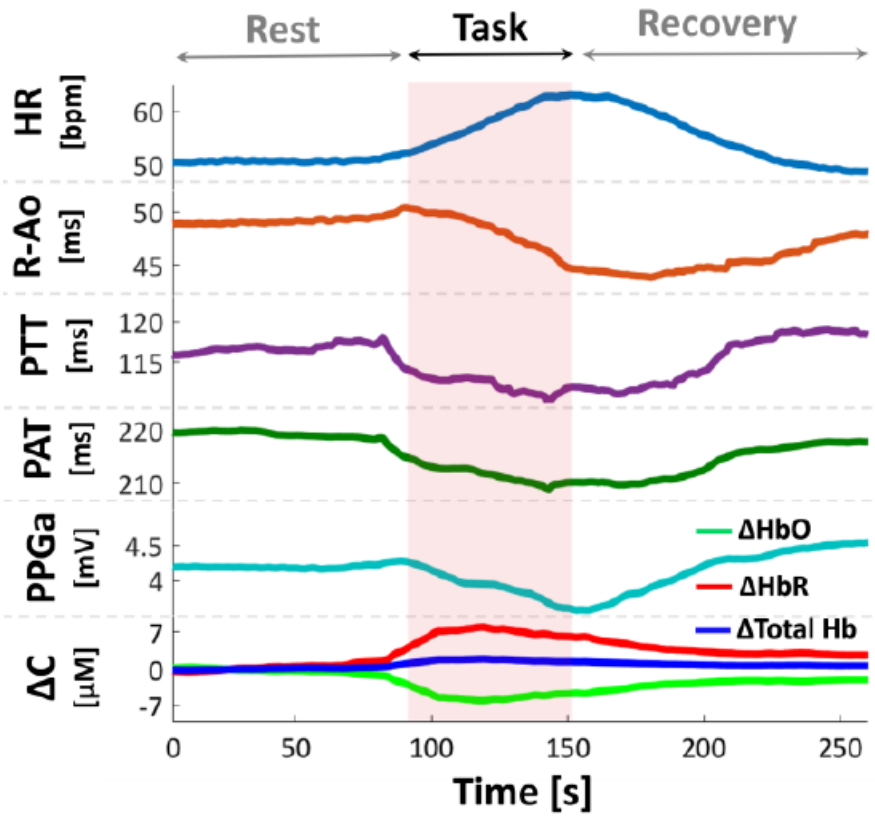

Figure 3-4. Extracted parameters for a subject transitioning from rest to mental stress. Changes in HR, R-Ao, PTT, PAT, PPG amplitude (PPGa), oxyhemoglobin (ΔHbO), deoxyhemoglobin (ΔHbR), total hemoglobin (ΔTotal Hb) were observed during stress. Data in figure were smoothed with moving average filter for better visualization. ΔC: Change in concentration, [M]: Molar.

for each subject, using the same classifier model and validation method [226]. A follow-up multiple comparison based on the Nemenyi Test was performed using the ranks generated by the Friedman Test [226]. A similar statistical analysis was performed on the RTLX scores as well to understand if the mental tasks induce significantly different workloads between the difficulty levels. For all analyses, p-values lower than 0.05 were considered statistically significant.

3.3.7 Results

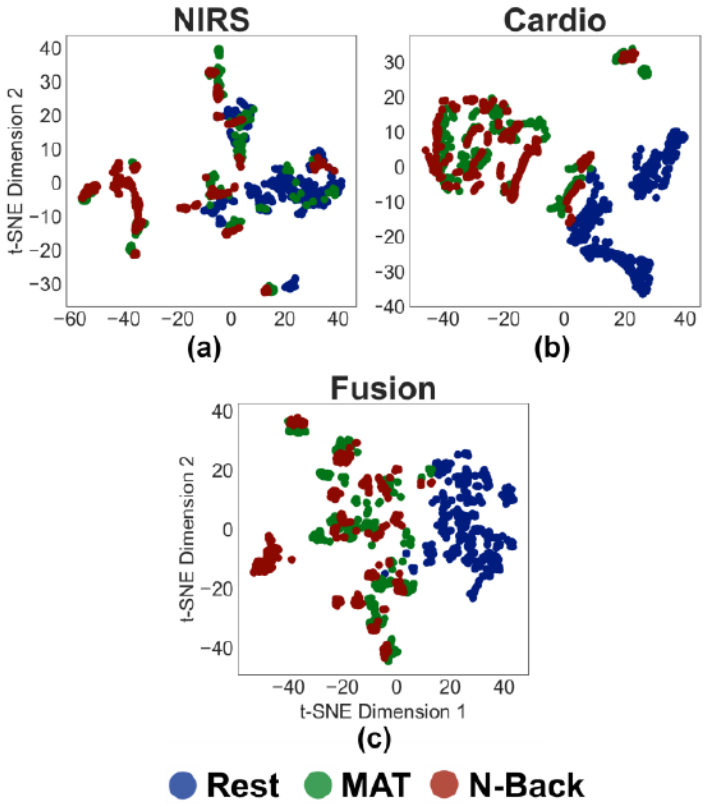

Figure 3-5. t-SNE plots for features related to a) NIRS sensing, b) peripherally measured cardiovascular sensing, and c) the fusion of both sensing modalities for rest, MAT, and N-back classes.

Figure 3.4 shows the extracted parameters for a subject transitioning from baseline rest to task. As the task starts, HR increases, R-Ao and PPG amplitude decrease, indicating increased cardiac and peripheral sympathetic activity. HbO, HbR, and Total Hb also show change during this transition, due to the change in oxygenation levels in the PFC. It should be noted that the directions in PTT, PAT, or concentration changes were not necessarily identical for each subject.

NASA-TLX Scores: The reader is referred to Figure 4 in Ref. [191] for RTLX boxplots. There are significant differences between the following intervals: easy-medium arithmetic ($p < 0.05$), easy-hard arithmetic ($p < 0.001$), easy-medium N-back ($p < 0.05$), medium-

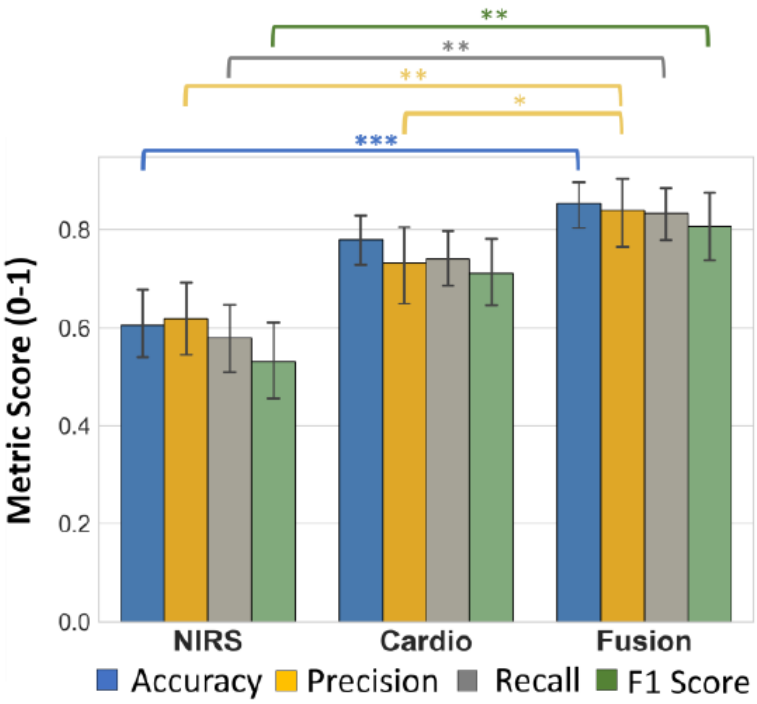

Figure 3-6. Summary of the performance metrics for classifier outputs for each sensing modality. * indicates statistical significance, *p < 0.05, **p < 0.001, ***p < 0.0001. Error bars:

hard N-back ($p < 0.05$), easy-hard N-back ($p < 0.001$). There is no statistically significant difference for task-wise comparisons of the same difficulty level (i.e. hard arithmetic versus hard N-back). These results suggest that the protocol was effective on different difficulty levels, compared to the rest.

Dimensionality Reduction from Selected Features: The reader is referred to Figure 5 in Ref. [191] for the t-SNE plots for NIRS features alone, cardiovascular features alone, and the fusion features to gain intuitive understanding for each sensing modality's ability to separate between classes (clusters). The plots were constructed from the features selected

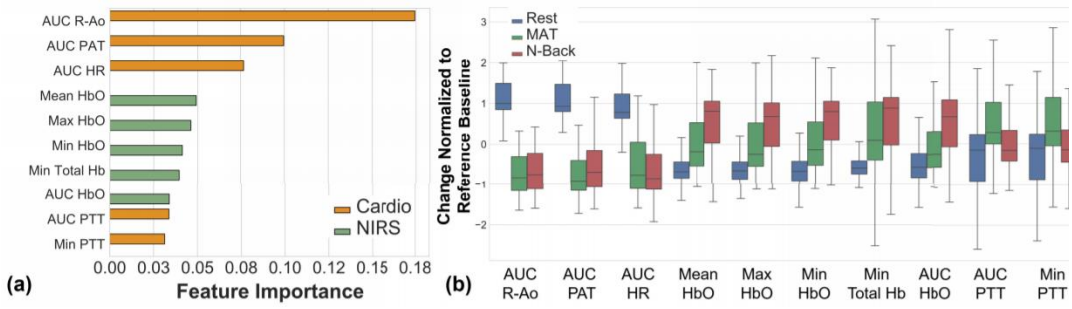

Figure 3-7. a) Feature importance ranking for the top 10 features. b) Boxplots of the top 10 features. Error bars: std.

by applying the univariate feature selection method to the whole dataset. The total number of selected features was 53. There were 23 NIRS features and 30 cardiovascular features.

Classification Results: Macro-averaged accuracy, precision, recall, and F1 scores for each class and sensing modality are shown in Figure 3.6. The fusion results in accuracy scores of 85% ± 9%, recall rate of 84% ± 14%, precision of 83% ± 10%, and F1 score of 80% ± 13% (mean ± SD). According to the Friedman and follow-up Nemenyi tests, accuracy ($p < 0.0001$), precision ($p < 0.001$), recall rate ($p < 0.001$), and F1 score ($p < 0.001$) significantly improve when fusion is used, as opposed to only NIRS. Moreover, there is significant improvement in precision from using only cardiovascular sensing to the fusion ($p < 0.05$).

Feature Importance Ranking: Figure 3.7a shows the importance ranking for the top 10 features. There are four cardiovascular-related features (R-Ao, PAT, HR, PTT), and five NIRS-related features (HbO and Total Hb). Figure 3.7b shows the boxplots of these features. The medians of the three cardiovascular features show notable difference between rest and other tasks (AUC R-Ao, AUC PAT, AUC HR). The

3.3.8 Discussion and Conclusion

In this work, enhancement in separating rest, arithmetic, and N-back tasks through the fusion of NIRS and peripherally measured cardiovascular sensing was investigated. Our hypothesis was supported through a custom designed NIRS-PPG headband along with cardiovascular measurements. Our results indicate that the fusion of sensors results in significant improvements in the classification of rest, arithmetic, and N-back tasks. The fusion of these sensing modalities looks compatible to merge the advantages of both worlds. Peripherally measured cardiovascular sensing represents a more central mechanism (due to the inclusion of the electrical activity and the mechanical motion of the heart), while NIRS sensing mainly target the PFC, which has an important role in working memory [227]. The improvements for accuracy, precision, recall rate, and F1 score from either of the sensing modalities to the fusion result from the ability to capture the PFC activity together with the central changes.

The significant enhancement for all performance metrics achieved via sensor fusion was particularly notable. Although the NIRS performance might arguably be improved by using multiple channels, using a single channel NIRS has its advantages for user convenience: multi-channel NIRS devices are often uncomfortable due to the size and high power requirements to feed multiple sources. A setup consisting of an accelerometer attached on the chest and a 3-lead ECG provide a simpler and practical means of data collection that would not block the forehead of the user.

Another remarkable result was the higher classification performance in all metrics between only cardiovascular and only NIRS sensing. Cardiovascular sensing outperformed NIRS

in the macro-averaged scores, although not statistically significant. This difference suggests that peripherally measured cardiovascular physiology might provide more consistent biomarkers of mental stress, compared to PFC activity biomarkers. A downside for using only cardiovascular sensing is that other types of stressors (i.e. temperature change or physical exercise) elicit cardiovascular responses similar to mental stress, thus, cardiovascular reactivity is not reliable across different stressors. For instance, physical exercise is shown to increase HR and decrease PEP (R-Ao) by multiple studies, resulting in the same directional changes as mental stress [228-231]. The addition of NIRS sensing by the fusion might rule out changes due to factors other than mental stress. Additionally, our results indicated improved precision from using fusion as compared to only cardiovascular sensing, highlighting that the fusion significantly improves the classification performance compared to using either of the sensing modalities alone. It should be noted that the other performance metrics, accuracy, recall rate, and F1 scores, were higher with the fusion, compared to using only cardiovascular sensing, although not statistically significant.

The interpretation of the t-SNE plots and boxplots of top 10 features seem consistent with clinical research on cardiovascular mental stress testing and the neuroscience literature: arithmetic tasks induce high cardiovascular reactivity [214], therefore cardiovascular sensing differentiates this type of stressor well, which appears as different median, compared to median of rest class for NIRS. Similarly, there is better separation between the medians of arithmetic and N-back NIRS features, when compared to cardiovascular features. This might be due to the high oxygenation activity in the PFC during N-back, as verified with comparison of multiple stressors in prior work [232]. The difference between

the sensitivity of the sensing modalities to each task class might be due to the different regions activated in brain during these tasks. Moreover, it could be anticipated that the fusion would enhance the classification of each mental task from the rest state (i.e. task versus rest classification). As expected from the clear separation in t-SNE plots, our model showed high classification scores for rest class. Specifically, we obtained macro-averaged scores of 98%, 99%, 98.5% for precision, recall, and f1 respectively. This indicates that our model could achieve highly accurate detection of mental stress (arithmetic or N-back) from the resting state.

According to the feature importance rankings, peripherally measured cardiovascular features have the highest importance for the classification (R-Ao, PAT, HR). These are followed with NIRS features and PTT. HR, a parameter always assumed to be vital in mental stress studies, does not have the highest ranking.

PAT and PTT were also selected in the top features, both of which were obtained by the SCG and head PPG signals. Since PAT contains both vasomotor-related (elevated blood pressure, decreased PTT) and contractility-related (elevated sympathetic activity, decreased PEP) influences, the contribution from both in the same direction might be the reason for the higher feature importance for PAT, compared to HR or PTT. Additionally, feature importance scores drop to below half for the NIRS features and PTT, highlighting the relative importance of the top three features. It should be noted that features related to PPG amplitude and ΔHbR were also among the selected features in each LOSO-CV loop, although they are not among the ten most important features. Mental stress affects the cardiac, vascular, and autonomic nervous system activity, hence the PPG signal stands as a rich source of physiological information as it is influenced by all these activities. Lastly,

the appearance of NIRS features (ΔHbO, ΔHbR, $\Delta Total\ Hb$) in selected features is not surprising, as multiple studies pointed out significant changes in the PFC oxygenation levels for different types of mental stressors [233].

An important strength of our methodology is the use of a statistical feature selection method (based on Benjamini - Hochberg p-value correction), for each LOSO-CV loop. Unlike manual feature selection, this method automatically selects the most useful features in each iteration. Another strength is in our validation scheme based on LOSO-CV, which is ideal for assessing future performance with naïve users. There is no need to collect calibration signals for a new user based on the methods described here.

One limitation of our current study is the size and homogeneity in demographics of the study population. In future studies, our methods may be validated with larger populations of subjects, and also will include persons with cardiovascular and neurological disorders. Another limitation is that the instrumentation requires placement of the sensors on multiple areas of the body. Future work can investigate the integration of the different sensing modalities—including both NIRS and cardiovascular signal acquisition—onto a single, head-worn device such as a headband.

Ease-of-use, accuracy, and length of training period are key criteria for HCI research and development. The addition of wearable hemodynamic measurements to NIRS sensing provides easy-to-use, higher performance HCIs that require no training for these types of mental stressors. HCIs could translate these physiological signals into a control signal for an external aid during the presence of such mental stressors, thus resulting in improved performance and successful augmentation of the human for challenging tasks.

3.4 Comparison of Autonomic Stress Reactivity in Young Healthy versus Aging Subjects with Heart Disease

The autonomic response to acute emotional stress can be highly variable, and pathological responses are associated with increased risk of adverse cardiovascular events. In this work, we evaluated the autonomic response to stress reactivity of young healthy subjects and aging subjects with coronary artery disease to understand how the autonomic stress response differs with aging [190].

Cardiovascular and behavioral responses to emotional stress have been associated with increased risk of primary [234-237] and secondary [238-241] coronary artery disease events. Furthermore, acute emotional stress events may trigger myocardial infarction and sudden cardiac death, but the mechanisms are not clear. In some studies, exaggerated mental stress reactivity is associated with increased future risk [242]. Paradoxically, blunted stress reactivity is also associated with increased risk [243]. This underscores the need for more research on the stress response in young, healthy groups and aging groups with CAD.

Prior studies have focused on stress-related changes in HR and BP in healthy subjects [228, 229, 231, 244, 245]. A subset of studies have also evaluated stress-related reactivity of specific cardiac markers, including ejection fraction and myocardial blood flow in patients with CAD [246-251]. The limitation of these metrics is that they do not provide specific autonomic pathways that would yield insight on targeted interventions. Studies which examine the physiologic measures specific to both the SNS and PNS changes with mental stress may provide more insight with regards to possible receptor-targeted therapeutics.

In this study, we examined two separate psychophysiology experiments with similar protocols to assess the stress reactivity in two different cohorts to evaluate the influence of age and coronary disease on stress reactivity. Aging and coronary disease are considered synonymous as they are strongly correlated: CAD is a manifestation of accelerated aging [252]. We consider aging as a progressive deterioration of physiological function, defined by a spectrum with two extremities: young, healthy subjects on one end, and aging patients with heart disease on another end. As CAD is an accelerator of biological age, aging patients with CAD and young, healthy subjects will represent the extreme ends of the aging spectrum. One cohort consisted of young (<30 years), healthy subjects, and the other cohort consisted of older (>55 years) subjects with CAD. In both cases, we examined stress reactivity to laboratory-induced arithmetic mental stress using SNS [253] and PNS (HF HRV) specific metrics. Additional standard metrics such as BP and HR were also assessed. We hypothesized that both PEP and HF HRV would decrease with mental stress in both groups to indicate sympathetic activation and parasympathetic inhibition, and the results would be exaggerated in the aging cohort vs. the healthy cohort as an indication of their worsened health status.

3.4.1 Human Subject Studies

Young healthy group: The study was performed under a protocol approved by the Georgia Institute of Technology Institutional Review Board (#H13512), at Georgia Institute of Technology, Atlanta, GA, between 01/07/2016 to 06/18/2016. A total of 25 adults (mean ± SD 25.4 ± 4.4 years, 5 females) were recruited through referral and written, informed consent was obtained. The subjects were students or staff without clinically apparent CAD or traditional risk factors for CAD.

Each healthy control subject was asked to relax for 15 minutes in a quiet, temperature-controlled (22˚C to 24˚C) room, after which the baseline resting signals were obtained. Mental stress was induced by a one-minute mental arithmetic test with serial addition paradigm, by the research staff. Negative feedback was provided for incorrect answers and delayed response times. Physiologic monitoring was conducted with 3-lead ECG, impedance cardiogram (ICG) and continuous BP during rest, stress, and recovery. ECG and ICG signals were collected using BN-EL50 and BN-NICO Amplifiers, Biopac Systems (Goleta, CA). BP was collected using Finapres (Enchede, Netherlands). All signals from the group of healthy subjects were collected at 2kHz sampling rate.

Aging group with CAD: A similar protocol to the healthy subjects was performed in a separate study, "Biofeedback in Mental Stress Ischemia" (ClinicalTrials.gov #NCT02657382), at Emory University School of Medicine, Atlanta, GA, between 03/30/2016 to 03/29/2017. A total of 25 adults (mean ± SD 64.8±5.9 years, 8 females) were recruited from the "Mental Stress Ischemia Mechanisms and Prognosis" cohort for a randomized study evaluating biofeedback vs. waitlist control, and written, informed consent was obtained [254]. Details of the inclusion exclusion criteria can be found elsewhere [254], but briefly, subjects with any history of CAD based on abnormal angiogram (>20% stenosis), nuclear stress test, or myocardial infarction were enrolled. The reader is referred to Figure 1 in Ref. [190] for the Consolidated Standards of Reporting Trials (CONSORT) diagram, comorbidities, medical history and medication usage of this cohort were listed in Table 1. Subjects who use beta-blockers were asked to hold usage in the morning before the procedure. As part of their evaluation in the Biofeedback study, mental stress reactivity was assessed during three separate visits, including pre-

intervention (vs. waitlist), post-intervention (vs. waitlist) at 8 weeks, and after waitlist intervention period (16 weeks), in which the wait-listed subjects received the intervention. Only the first per subject visit was evaluated for this analysis to allow for independent sampling.

Similar to the healthy group study, each aging group subject was asked to relax in a temperature-controlled room. A three-minute math serial subtraction paradigm was conducted with negative feedback, by the research staff. ECG and ICG were collected using the VU AMS Ambulatory Monitoring System (Amsterdam, Netherlands), at a sampling rate of 1 kHz. Noncontinuous systolic/diastolic BP values were collected using an automatic Omron BP monitor in every 0, 5, 25 and 30 minutes of rest and each one minute of stress.

For this group of subjects, the relationship of CAD severity and stress reactivity was also quantified using Gensini scores on their last angiogram. The Gensini scores quantify CAD burden from the extent and severity of the coronary artery involvement [255, 256]. The Gensini scores were computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its importance based on location. Linear models estimated the change in physiological measures with stress that corresponded to the Gensini scores for each respective subject.

3.4.2 Signal Processing

All signals were processed using MATLAB 2016a (Natick, MA). The ECG was bandpass filtered (10–25 Hz, linear, finite impulse response (FIR)) to exaggerate QRS complex for feature extraction and noise reduction. Similarly, the ICG (dZ/dt) was bandpass filtered

(0.8- 20Hz, linear, FIR) for noise reduction. Since all subjects had normal sinus rhythm, ECG R-peaks were detected with thresholding to calculate HR and HRV, and edited, if needed, for erroneous peak detection due to noise. For HRV, the non-constant R-R intervals were interpolated using piecewise cubic hermite interpolating polynomial to obtain constant intervals between R-peaks, to be able to compute power spectral density (PSD). The area under the PSD curve from 0.15 Hz-0.4 Hz was extracted as HF HRV. The standard deviation of HR (std HR) was also calculated as a secondary measure. PEP measurement started with the detection of the onset of myocardial depolarization. This onset was used as a reference for the beginning of each myocardial heartbeat cycle. The filtered ECG signal exaggerated the onset of R-peak and S-peaks, such that the QRS complex was distinguishable with two negative peaks that correspond to R-peak onset and S-peak, respectively. To detect the onset, first the filtered ECG signal was inverted, and both R-peak onset and S-peak were found by thresholding (two peaks per beat). Then, the odd-numbered peaks were selected, which correspond to the onset. Manual review of peak detection was also performed to exclude areas with artifact. The R-peak onsets were used to segment all the signals into individual beats.

To reduce the effects of motion artifacts on the individual, segmented ICG beats, exponential moving averaging of successive beats was implemented with a 10-beat time constant.

PEP was calculated as the difference between the onset of myocardial depolarization and the opening of aortic valve. The B-point on the dZ/dt waveform corresponds to the opening of aortic valve, however, its mathematical detection is not well-defined in the literature. Although it is a physiological event occurring in every single cardiac cycle, the notch or

inflection point defining the B-point is not always apparent [257, 258]. Several mathematical algorithms have been developed for the automatic estimation of B-point [257], and there are discrepancies between methods in different experimental conditions [259]. Recently, three popular B-point detection algorithms have been compared, and the algorithm based on the third derivative of ICG was validated to perform significantly better [260]. Therefore, in this work, the third derivative approach was used to detect B-point. Specifically, the global maxima (dZ/dt_{max}) was first calculated (C-point), which represents maximum left ventricular flow. Then, the maximum of the third derivative (dZ^3/dt^3) of the ICG signal before the C-point was calculated as the B-point. Therefore, PEP was calculated as the difference between the ECG myocardial depolarization onset and ICG B-point. Continuous BP signal was not smoothed further, since BP device applies post-processing. Continuous BP physiological markers were calculated by first ensemble averaging the BP beats, referenced by the R-peak onset. The peaks and valleys of each beat correspond to systolic (SBP) and diastolic (DBP) blood pressures, respectively. Pulse pressure (PP) was calculated by measuring the difference between SBP and DBP.

3.4.3 Statistical Analysis

Stress reactivity was evaluated by comparing the first minute of stress with a one-minute rest period that started five minutes prior to stress-onset. Only one minute was evaluated for both cohorts, because the stress duration in the healthy cohort was one minute. To affirm whether significant hemodynamic changes occur from the first minute of stress to warrant its use for the aging group with CAD (instead of the mean for the entire stress period), a supplementary analysis was performed using the vital measures HR, SBP, DBP, PP to evaluate changes from baseline to the first minute of stress and mean overall stress,

detailed in [190]. Statistical significance was tested with a one-way ANOVA for normally distributed data, or Kruskal-Wallis for non-normal data. Later, follow-up parametric or non-parametric multiple comparisons tests based on Tukey-Kramer honest significant difference criterion were performed to understand which intervals significantly differ from each other. Stress reactivity was measured by subtracting stress values from rest values. A paired t-test was used to compare stress versus rest. Cohen's distance was calculated using the mean of two states for each group, and converted to effect size. A two-sample t-test for unequal variances was used to compare the stress-to-rest ratios between the cohorts. The evaluation for covariate effects was limited due to the small sample sizes. Nonetheless, because of the potential role of sociodemographic and health factors, an exploratory analysis was performed to evaluate for group differences by risk factor. Linear regression models were performed to adjust for age, sex, beta-blocker usage, angiotensin-converting-enzyme (ACE) inhibitor usage, and antidepressant usage when calculating group-level differences. The proportion of subjects whose physiological values increased or decreased with stress was also tabulated as a secondary method of evaluating stress reactivity; for this, Chi-square or Fisher's exact test was used to detect group differences, where appropriate. For all statistical analyses, p-values less than 0.05 were considered significant. All statistical analyses were carried out using MATLAB Statistics and Machine Learning Toolbox.

3.4.4 Results

In the healthy group, 80% were male and the mean (SD) age was 25.4 (4.4) years. In the aging group, 68% were male and the mean (SD) age was 64.8 (5.9) years. Table 3.1 presents the mean, standard error of the mean, percent changes, confidence intervals (CI),

p-values, effect sizes of the physiological markers extracted from ECG, ICG, and BP signals for each study, for rest and stress states. Due to device malfunction one subject, BP signals were collected from 24 patients. As the primary outcomes, PEP decreased significantly for both groups, while HF HRV decreased significantly only in the aging group.

The healthy group showed 50% higher PEP decrease (9.6ms) than the aging group (6.4ms) during mental stress. In total, five physiological markers (HR, PEP, SBP, DBP, PP) were found to be significantly different between stress and rest for the healthy cohort (n = 25). Five physiological markers (HR, PEP, SBP, PP, HF HRV) were also found to be significantly different between stress and rest for the aging cohort (n = 24 for BP levels, n = 25 for other markers).

No statistically significant or large effect size difference ($r < 0.4$) in stress reactivity was found between groups, regardless of adjustment for age, sex, beta-blocker usage, ACE

Table 3-1. Data for both groups: Rest, stress and mean of differences.

	Healthy Group (n = 25)						Aging Group (n = 25¶)					
	Rest	**Stress**	**S-R**	**95% CI**	**P**	**r**	**Rest**	**Stress**	**S-R**	**95% CI**	**P**	**r**
HR [bpm]	92.9 (2.5)	101.3 (3.4)	8.4*	(3, 13.7)	<0.01	0.28	62 (1.8)	69.4 (2.5)	7.4*	(4.1, 10.6)	<0.001	0.32
PEP [ms]	109.3 (3.9)	99.7 (4.3)	-9.6*	(-14.2, -5.1)	<0.001	0.23	80.9 (4.3)	74.5 (3.8)	-6.4*	(-10.9, -2)	<0.01	0.16
HF HRV [log-ms^2]	2 (0.1)	1.9 (0.1)	-0.1	(-0.4, 0.2)	0.71	0.1	2.6 (0.2)	2.3 (0.2)	-0.4*	(-0.6, -0.1)	0.01	0.21
SBP [mmHg]	117.4 (3.5)	128.5 (3.9)	11*	(8.3, 13.7)	<0.001	0.29	130.4 (3.7)	147.4 (6.1)	17*	(10.7, 23.2)	<0.001	0.22
DBP [mmHg]	75.9 (1.7)	81.6 (1.8)	5.7*	(4.3, 7.1)	<0.001	0.31	74.8 (2)	77.8 (2.7)	3	(-0.2, 6.2)	0.09	0.08
PP [mmHg]	41.6 (2.1)	46.9 (2.4)	5.3*	(3.5, 7.2)	<0.001	0.23	55.6 (2.9)	69.6 (4.4)	14*	(9, 19)	<0.001	0.3
std HR [bpm]	5.6 (0.9)	5.9 (0.5)	0.2	(-1.4, 1.9)	0.79	0.49	4 (0.5)	3.8 (0.5)	-0.1	(-1.1, 0.9)	0.82	0.02

Values represent mean (SEM). * Denotes change in feature from rest to stress is significant, $P \leq 0.05$. ¶Denotes n = 24 for SBP, DBP, PP. S-R: Mean of differences between states, calculated by stress-rest for each subject for the corresponding feature. 95% CI: 95% Confidence Interval (Lower bound, Upper bound). P: P-value. r: Effect size.

inhibitor usage, or antidepressant usage. We also explored the possibility of gender-related bias in healthy population: within the young group, there is no statistically significant or meaningful effect size difference between the stress responses between young men and

Table 3-2. Comparison of ANS activity biomarkers.

ANS Activity Indicators	**Healthy (n = 25)**	**Aging (n = 25)**	**Difference**
HR increase	72%	80%	8%
PEP decrease	88%	80%	8%
HF HRV decrease	56%	80%	24%

Values represent the percentages of each population (n = 25 healthy subjects, n = 25 aging subjects).

young women in any of the measured parameters. Table 3.2 compares the directionality of physiological changes from rest to stress. For the healthy cohort, 72%, 88%, and 56% of the subjects showed increased HR, decreased PEP, and decreased HF HRV, respectively.

The corresponding percentages were all 80% for the aging cohort. The proportion of subjects with a decrease in HF HRV was 24% less in the healthy cohort compared to the aging cohort ($p = 0.08$).

Figure 3.8 summarizes the distributions of the stress-minus-rest values for HF HRV and PEP. Most subjects in both groups showed decreases in both values, consistent with increase in SNS and decrease in PNS during stress. No statistically significant differences were found between groups in individual quadrants.

3.4.5 Discussion and Conclusion

This study represents the first attempt, to our knowledge, of assessing cardiac SNS and PNS mental stress reactivity in healthy versus aging subjects with CAD; to our surprise, we found a large proportion (44%) of subjects in the healthy group had paradoxical

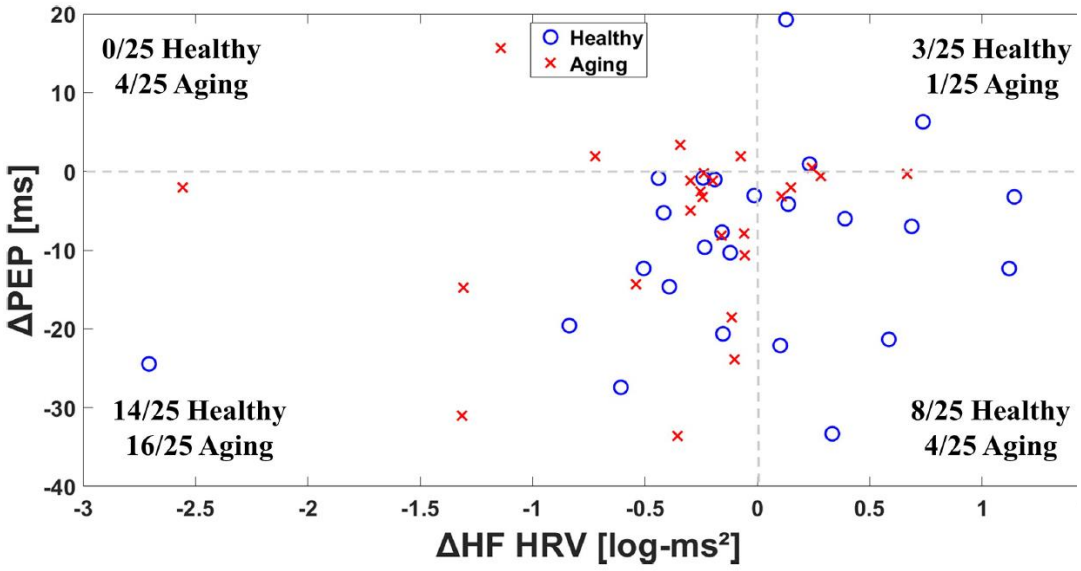

Figure 3-8. Changes in HR HRV and PEP responses, shown as ΔHF HRV vs. ΔPEP. Values show difference between stress and rest, as stress-rest. Each quadrant shows the number of aging and young/healthy subjects.

increases in HF HRV, a marker of PNS activity, and a 20% of subjects in the aging group demonstrated decreases in PEP, a marker of SNS reactivity. While the differences between groups were not statistically significant, the nature of this study was exploratory. Overall, this study warrants further evaluation of physiologic stress reactivity as it relates to aging and disease, and supports the notion that, in many cases, the expected increase in SNS and decrease in PNS with mental stress challenge do not occur. This may be related to pathological reasons, like myocardial ischemia (which would increase PEP) or reflexive PNS activation (which would decrease HF HRV).

The increased PNS activity in response to mental stress that was largely noted in the healthy group is potentially adaptive. It may be a compensatory autonomic reflex to the sympathetic stress response, as a way to buffer against the pathophysiologic effects of sympathetic activation. Such mechanisms may perhaps be disrupted in subjects with CAD and explain the lower proportion of CAD subjects who demonstrated an increase in PNS: cardiac PNS activity was previously studied to decrease with age [261], and it is known that there are histological and functional reductions in sinus node during aging [262-264]. Aging populations are more likely to have sinus node dysfunction, which decreases their PNS regulation ability through sinus node [265], as they are more susceptible to stress effects. Young people, on the other hand, would have better PNS regulation through sinus node as there is not as much deterioration.

The increased PNS activity in some young healthy adults might be due to this respiratory-activated PNS activity. Young individuals may also have more intact baroreflex mechanisms, which activate the PNS in response to increased blood pressure. This may

have occurred since the younger group was observed to have a hypertensive response during stress. Aging group with CAD experienced significant decrease in HF HRV likely because the sinus node activity or baroreflex mechanisms were impaired. Although mean PEP decreased, a small percentage of each group showed increased PEP with stress. In the aging cohort who had decreased HF HRV to indicate PNS withdrawal, prolonged PEP may be a consequence of impaired mechanical contraction secondary to ischemia, transient conduction disease, or other causes [266-268]. Impaired baseline left ventricular function was also noted in one of the subjects in the aging group with increased PEP. It may also suggest that the arithmetic challenge did not induce significant mental stress. Overall, these paradoxical PEP increases during mental stress warrants further investigation and may suggest increased risk.

The reasons for potential differences cannot be attributed to any single factor. The aging group had high prevalence of hypertension (68%), dyslipidemia (80%) and diabetes (40%); 48% of this cohort also had history with smoking. These conditions, in addition to CAD, may have contributed to the decrease in HF HRV with mental stress as well. Medications may also explain a difference in HF HRV between groups, although the aging group was asked to withhold beta-blockers in the morning before the procedure, which are one of the main potential confounders.

Differences in baseline blood pressure and its stress reactivity were noted, with higher values in the aging group. Such differences are consistent with prior literature [269-271]. The group difference in pulse pressure are the most notable; the mean change from stress to rest (14mmHg) in the aging group was nearly three-fold higher than the mean change in healthy group (5mmHg). In multivariable models, the differences were not significant after

adjustment for age and sex, however. Short-term changes in blood pressure are mainly controlled by the baroreflex, which is one of the major determinants of SNS activity [192]. Aging subjects have higher blood pressure (SBP, DBP, PP) during baseline and mental stress, compared to the young healthy group, and their stress responsivity in terms of SBP and PP is higher, consistent with previous studies [272]. The three-fold difference in PP between groups signal that age-related changes in baroreceptors increase sympathetic responsivity to mental stress.

This study is subject to limitations. We compared stress reactivity from two independently conducted studies in an exploratory analysis. We felt this was justified because both utilized arithmetic stressor tasks, and the majority of the findings are in agreement with the literature and credible biological concepts. We restricted our analysis to the first minute of stress only, and this was justified by findings that stress reactivity was consistently higher in the first minute compared to the entire stress period. The reader is referred to S1 Table in Ref. [190] . As per Table 3.1, HR and SBP responses between groups were similar between studies, which suggest a similar overall net stress responsivity with regards to some summative measures. On the other hand, differences in DBP reactivity can be explained by differences in vascular stiffness that occur as a part of aging [273]. Confounding from CAD may have also played a role, although we found that within the aging group, CAD burden (Gensini score) did not associate with stress reactivity, and therefore unlikely to be a confounder. S2 Table in [190] shows the related data. Nonetheless, the results could be strengthened if the study included an older group without CAD. Data on such subjects have been published elsewhere; a group of older participants with mean (SD) age of 67 (1) underwent Trier Social Stress Test and demonstrated a similar

heart rate response [274]. In another study of CAD patients versus healthy controls, CAD status did not associate with changes in heart rate reactivity to stress [275]. Although our older group has CAD and other comorbidities, as well as medication exposures, our sample size does not adequately allow comparisons between groups or within groups. Therefore, we describe the differences between groups due to aging, which is nonspecific, but nonetheless summarizes the large number of comorbidities that are usually associated with the aging process (such as hypertension) [276]. Despite the small sample size, working between two extremities in age/health status increases the likelihood of capturing the group differences. Another limitation is that all women in the aging group were post-menopausal, and as such we could not evaluate the effects of this specifically. Menopause is an important aspect of aging in women, and may have had an independent effect on stress reactivity in the women of the aging group response (i.e. increase in stress response as observed by) [274]. We were able to only report minimal HRV metrics because of the minimum ECG signal length for the spectral analysis of HRV. Frequency-domain HRV analysis requires approximately one minute of ECG recording for assessing high frequency HRV (> 6 cycles), and two minutes of recording for assessing LF HRV [195]. HF HRV reflects changes in the PNS activity, whereas LF HRV is influenced by both SNS and PNS activity. The ratio of both powers, LF/HF HRV, is widely adopted in the literature to be an indicator of sympathetic tone, however multiple studies proved its inconsistency to reflect sympathetic control on the heart [196, 203]. As we were primarily interested in PNS influences during the one-minute mental stress testing in our study, we only reported HF HRV results.

Our findings underscore the need for multiple physiologic monitoring methods to assess the effects of acute mental stress on cardiovascular physiology. Although ECG is commonly measured, ICG is less commonly employed. For ambulatory studies, this can be more difficult, although new devices allow for real-time monitoring of stressful situations. PEP is an underused measure in clinical studies due to the requirement for ICG, however its use to reflect the SNS activity was studied by many groups: a comparison of PEP and LF/HF HRV showed that PEP outperforms LF/HF for conditions that are known to increase sympathetic activity, or LF/HF is specifically shown unable to reflect changes in SNS activity, such as mental stress [277], exercise [278], and beta-adrenergic/cholinergic blockade [279].

In summary, we performed an analysis of SNS and PNS responses to acute mental stress in healthy and aging subjects. PEP decreases with stress regardless of health and age status, as anticipated. Contrary to what we expected, we found that many (44%) healthy subjects did not experience vagal withdrawal, and 20% of aging subjects demonstrated paradoxical increases in PEP. The differences between groups are not statistically significant as our sample size is limited. The fundamental limitation of our study is small sample size, but the utility of the study is the preliminary data that it provides for the larger investigation that is based both on the data, and the fundamental aspects in biology. The results highlight possible limitations of these measures in psychophysiology studies, as well as the need to include multiple complementary autonomic measurement modalities in clinical investigations.

This chapter highlighted approaches to quantify autonomic nervous system activity and validated these approaches by reporting three independent datasets related to mental stress

in human subjects with or without heart disease. These methodologies laid the groundwork for investigating the effects of nVNS in stress in human subjects, which is detailed in Chapter 4.

CHAPTER 4. TRANSCUTANEOUS CERVICAL VAGUS NERVE STIMULATION AS A NOVEL THERAPY FOR STRESS AND PTSD

4.1 Introduction

This chapter discusses the techniques and methods used for a sham-controlled double blind randomized human subjects study that investigates the effects of transcutaneous cervical vagus nerve stimulation (tcVNS) in stress and PTSD. The study was designed as a multi-dimensional study, with investigations in physiological effects, biochemical biomarkers, and brain imaging. The scientific outcomes focus on different investigations with various analyses, and engineering outcomes focus on real time physiological biomarkers of tcVNS and their potential for closing the loop for personalized neuromodulation and titration of therapy. These biomarkers could be instrumental in a machine learning guided neuromodulation scheme, applicable outside of clinical settings.

4.2 Sham-Controlled Double Blind Randomized Human Subjects Study

4.2.1 Human Subjects Study

The study was performed under a protocol approved by the institutional review boards of Emory University (#IRB00091171), Georgia Institute of Technology (#H17126), The Space and Naval Warfare (SPAWAR) Systems Center Pacific, and the Department of Navy Human Research Protection Program. The study took place in Emory University School of Medicine between May 2017 and October 2019 (ClinicalTrials.gov #NCT02992899). Participants included physically healthy adults between ages 18-65 with a history of psychological trauma. For the investigation of physiological biomarkers, participants who

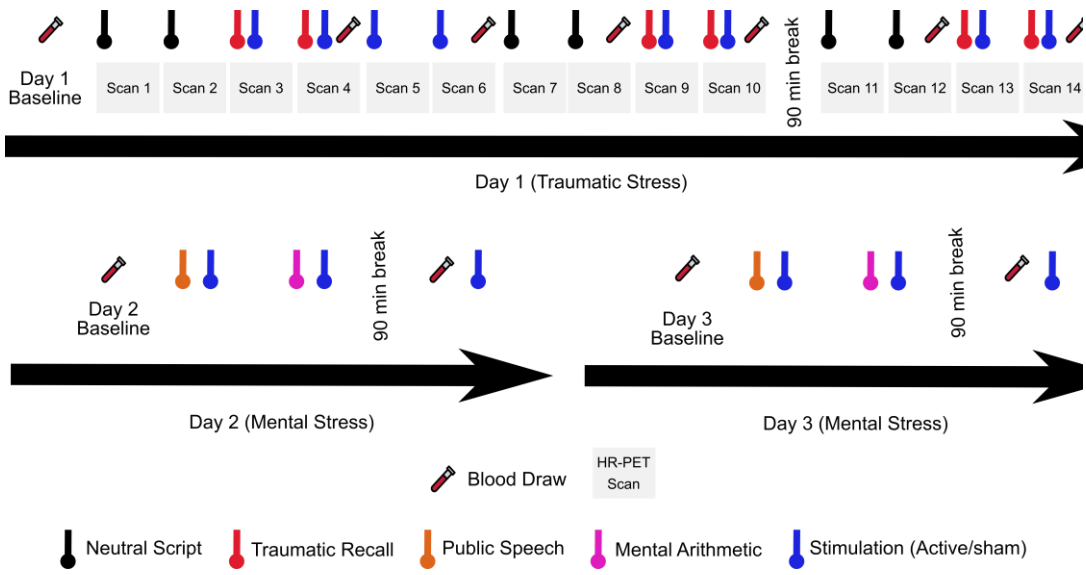

Figure 4-1. Protocol timeline depicting neutral and trauma scripts, HR-PET scans (first day), mental stress (second and third day), and blood draws (all days).

met criteria for PTSD were analyzed separately from participants who did not meet criteria for PTSD (Non-PTSD). For blood biomarker investigations, analyses were completed as either together or separate, where appropriate, due to limited sample size. Exclusion criteria were: pregnancy; meningitis; traumatic brain injury; neurological disorder; organic mental disorder; history of loss of consciousness greater than one minute; alcohol abuse or substance abuse based on the Structured Interview for DSM (SCID) [280], within the past 12 months; current or lifetime history of schizophrenia, schizoaffective disorder, or bulimia, based on the SCID; a history of serious medical or neurological illness, such as cardiovascular, gastrointestinal, hepatic, renal, neurologic or other systemic illness; evidence of a major medical or neurological illness on physical examination or as a result of laboratory studies; active implantable device (i.e. pacemaker); carotid atherosclerosis; cervical vagotomy. CAPS was administered to evaluate for presence and severity of both possible current and lifetime PTSD [40]. The protocol consisted of three subsequent days

for each participant, Figure 4.1 presents the details for each day. Participants were instructed to withhold any stimulant (i.e. coffee) throughout the entire protocol. Each participant was asked to write their traumatic events; later, personalized voice recordings based on these scripts were prepared. The first day included six traumatic recall scripts and six neutral scripts presented audibly through headphones to participants inside a high-resolution positron emission tomography (HR-PET) scanner at 20°C temperature, starting approximately at 8AM. On day 1, participants were prepared with noninvasive sensing modalities, dedicated neuromodulation devices, and a headphone by the researchers. The protocol started when they lay down in a high-resolution positron emission tomography (HR-PET) scanner bed (head inside the scanner) for 14 scans, each took approximately eight minutes. Before the scans started, baseline physiological data were collected in the same posture. In the first two scans, "neutral" pleasant scenery recordings (for imaging purposes, without stimulation) were delivered audibly. In scans three and four, traumatic stress recordings were delivered immediately followed by stimulation. In scans five and six, stimulation was applied in the absence any acute stressor. From scans seven to ten, two neutral recordings (no stimulation) and two traumatic stress recordings followed by stimulation were applied. Then, the participants took a 90-minute break. After the break, four more scans were taken that included two neutral recordings and two traumatic stress recordings followed by stimulation, respectively. In short, the first day included audible delivery of six neutral scripts, six traumatic stress scripts followed by stimulation, and two stimulations without acute stress in 14 HR-PET scans. All neutral/traumatic recordings were approximately one minute in duration. The second and third days were the same as each other: they did not include brain imaging and they focused on non-personalized

mental stressors. Baseline signals were recorded from the participants, and they underwent a public speech and a mental arithmetic task, both immediately followed by stimulation. In the public speech task, the participants were given two minutes preparation time to prepare a defense statement in a scenario they were accused of theft at a shopping mall. Their speech was immediately followed by stimulation. Following eight minutes in silence after stimulation stopped, the participants were required to answer a series of arithmetic questions as fast as possible for three minutes. Immediately after the arithmetic task, another stimulation was applied, and the participants waited for eight more minutes in silence (post-stimulation period). For both mental stress tasks, negative feedback was provided for incorrect answers and delayed response times to exaggerate the stress effect. After this mental stress paired with stimulation paradigm, a 90-minute break was given. After the break, a third stimulation was applied without any acute stressor. All days included baseline and protocol blood draws, as indicated in Figure 4.1.

4.2.2 Baseline Assessments

Each subject informed, provided written consent, after which they underwent a psychological and health assessment. Sociodemographic factors (age, sex, race/ethnicity, marital status, education level) and clinical information (current medications, medical history, SCID) were collected. Psychological assessment was performed using structured interviews and relevant questionnaires, including PTSD Checklist-Civilian Version (PCL-C) [281], PTSD Symptom Scale (PTSD-SS) [282, 283], Clinician-Administered PTSD Scale (CAPS, only patients diagnosed with PTSD qualifies for CAPS) [40], early trauma inventory (ETI) [284], adulthood trauma inventory (ATI) [285], Hamilton Anxiety Scale (HAM-A) [286], Hamilton Depression Scale (HAM-D) [287], Beck Depression Inventory

(BDI) [288], Social Support (ESSI) [289], and Physical Activity (Baecke Questionnaire's Work, Sports, Leisure indices, respectively) [290].

4.2.3 Blinding

The participants were randomized into either active tcVNS or sham stimulus groups with an online randomizer using simple randomization. The devices were pre-numbered by the manufacturer who were not involved in the research, and random allocation was conducted by an individual who did not take part in enrollment, data collection or analysis. Enrollment was done by clinical staff. The participants and clinical staff were blinded to the stimulus type, and each of the participants received only one type of stimulus. The researchers, who were also blinded to the stimulus type, conducted the questionnaire assessments, data collection, signal processing, and parameter extraction. Stimulus grouping (active or sham) was un-blinded for the interpretation of statistical analysis.

4.2.4 Active and Sham Stimulation

Both active tcVNS and sham stimuli were administered using hand-held devices (GammaCore, ElectroCore, Basking Ridge, New Jersey) with identical placement and operation. tcVNS or sham was applied using collar electrodes on the left side of the neck. The treatment area on the neck was located by finding the pulse on the carotid artery for each participant (Figure 4.2). Conductive electrode gel (GammaCore, ElectroCore, Basking Ridge, New Jersey) was used to maintain good contact between the skin and the electrodes. Active tcVNS devices produce an AC voltage signal consisting of five 5kHz sine pulses, repeating at a rate of 25Hz. Sham devices produce an AC biphasic voltage signal consisting of 0.2Hz square pulses that delivers a mild buzzing sensation similar to

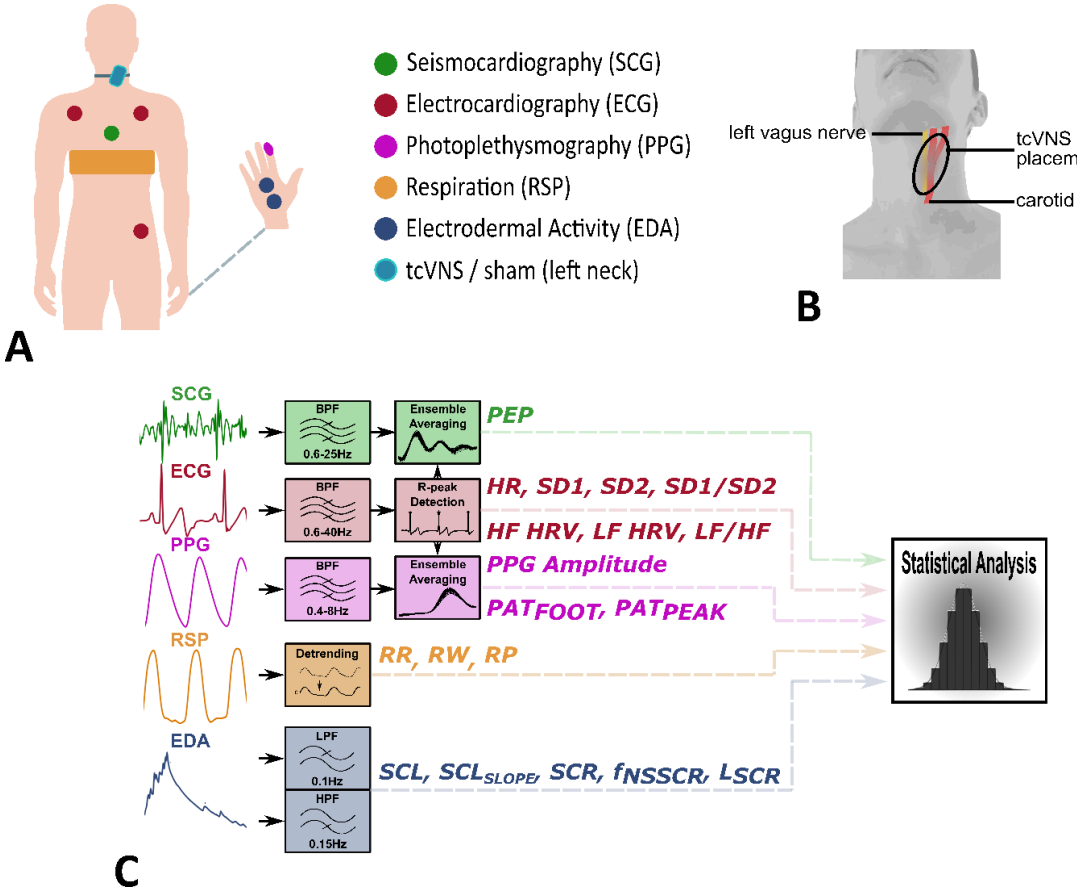

Figure 4-2. Data collection and signal processing summary. a) Non-invasive sensing modalities shown on participant, active or sham stimulation was applied from left neck. b) Representation of relative locations of left carotid arteries and left vagus nerve. tcVNS electrodes were placed onto the area where the carotid pulsation was located. c) Signal processing and feature extraction summary.

the active device. Both active and sham device operation stops automatically after 120 seconds. The stimulation intensity was adjustable using a roll switch that ranged from 0 to 5a.u. (arbitrary units) with a corresponding peak output ranging from 0 to 30V (~ 0 to 60mA) for active tcVNS, and from 0 to 14 V (~0 to 60mA) for sham device. During each application, the stimulation intensity was increased to the maximum the participant could tolerate, without pain. At the start of stimulation, the intensity was increased gradually until each participant instructed to stop. The stimulation continued at the selected intensity.

4.2.5 Active versus Sham Stimulation Rationale

High frequency voltage signals (such as the active stimulus) pass through the skin with minimal power dissipation due to the low skin-electrode impedance at kHz frequencies; in contrast, lower frequency signals (such as the sham stimulus) are mainly attenuated at the skin-electrode interface due to the high impedance [291]. Accordingly, the active device operating at higher frequencies can deliver substantial energy to the vagus nerve to facilitate stimulation, while the voltage levels appearing at the vagus would be expected to be orders of magnitude lower for the sham device and thus vagal stimulation is highly unlikely. Nevertheless, since the sham device does deliver relatively high voltage and current levels directly to the skin, it activates skin nociceptors, causing a similar feeling to a pinch. This sensation is necessary for blinding of the participants, and was thought as a critical detail by the researchers for the valuation of the potential treatment in psychiatric populations. In the current study, no participant reported an absence of sensation for any application. In another study, an active stimulation amplitude higher than 15V using the studied device was reported to create vagal somatosensory evoked potentials associated with vagal afferent activation reliably, that are also activated with VNS implants [175].

4.2.6 Physiological Monitoring

Physiological data were collected by the measurement of ECG, PPG, SCG, EDA, RSP, and BP, Figure S3a details the electrode placement for each participant. Wireless 3-lead ECG and piezoresistive strap-based RSP were collected through RSPEC-R amplifiers; transmissive, index finger-based PPG and inner palm-based EDA were collected through PPGED-R amplifiers from Biopac Systems (Goleta, CA). A low noise 356A32

accelerometer (PCB Electronics, Depew, NY) was taped with a Kinesio tape on mid-sternum for SCG monitoring, with Z-axis surface touching the sternum, aligning with the dorsoventral movement of the heart. For EDA measurement, an isotonic electrode gel (GEL101) and pre-gelled isotonic electrodes (EL507) were used (Biopac Systems, Goleta, CA). Continuous ECG, PPG, SCG, EDA, and RSP data were simultaneously transmitted to a 16-bit MP150 data acquisition system at 2kHz sampling rate (Biopac Systems, Goleta, CA). Non-continuous, cuff-based SBP and DBP values were recorded periodically with an Omron blood pressure cuff for baseline, stress, stimulation, and post-stimulation intervals.

4.2.7 Signal Processing and Parameter Extraction

The signal processing and parameter extraction were carried out in MATLAB (R2017b, Natick, MA). The following parameters were extracted: heart rate (HR), pre-ejection period [253], amplitude of PPG, PAT (PAT_{FOOT}, PAT_{PEAK}), RR, RW, RP, LF HRV, HF HRV, SCL, SCR, frequency of non-specific skin conductance responses (f_{NSSCR}), and latency of skin conductance response (L_{SCR}). Figure 4.2c shows sections from collected physiological signals from a participant and the parameters computed from these signals. The ECG, SCG and PPG signals were filtered with finite impulse response band-pass filters, with cut-off frequencies 0.6-40Hz for ECG, 0.6-25Hz for SCG, and 0.4-8 Hz for PPG, respectively, to preserve the waveform shape and cancel the noise outside their bandwidths. The phasic component of EDA (for computing the parameters related to skin conductance response) was obtained using an FIR 0.15 Hz equiripple high-pass filter. The slowly varying RSP signal was used as is, as the module applies 10Hz low-pass filter internally. The R-peaks of the ECG signals were detected using thresholding, and were used to calculate HR, HRV. SCG and PPG signals were ensemble averaged according to

the R-peaks, using beat lengths of 150ms for SCG and 600ms for PPG. These lengths were sufficient to detect the fiducial points of each SCG and PPG beats. To reduce the effects of motion artifacts on the individually segmented beats, exponentially weighted moving ensemble averaging of successive beats was implemented for some parameters described below.

Pre-Ejection Period: R-Ao (i.e., PEP) values were computed following a three-beat exponential moving averaging procedure for noise reduction.

PPG Amplitude and Pulse Arrival Times: As a measure of peripheral sympathetic activity and vasomotor activity at the area of signal collection (index finger), the amplitude of each PPG beat was extracted. Second, PAT was calculated from two reference points with a time constant of five beats [85]. The first reference point was the foot of PPG signal, which was located by finding the maximum of the second derivative of the pulse wave before the maxima (PAT_{FOOT}). The second reference point was the peak (maxima) of the PPG signal (PAT_{PEAK}).

Respiratory Measures: Due to the loosening of the respiration belt over time while the participant was inside the PET scanner, the respiration signal occasionally had a DC offset. To remove this offset, a sixth order polynomial was fit to the signal in each interval (i.e., rest or stress), and the signal was detrended. From the detrended signal, the peaks representing inhalation and exhalation were located using thresholding. The rate of the peak appearance was extracted as RR. For RW, the width of each peak was computed as the distance between the points to the left and right of the peak, where the descending signal intercepts a horizontal reference line. The reference line was positioned beneath the peak

at a vertical distance equal to half the peak prominence. The points themselves were found by linear interpolation. RP measured the prominence of a peak, i.e. how much the peak stands out due to its intrinsic height and its location relative to other peaks. It was calculated as the minimum vertical distance that the signal descends on either side of the peak before either climbing back to a level higher than the peak or reaching an endpoint.

Heart Rate Variability Measures: Two techniques were used to extract multiple HRV measures: Frequency-domain analysis and joint time-frequency analysis (Poincaré method). For both frequency-domain HRV and Poincaré analyses, ECG signals from the start and end of the days (longer than five minutes), ECG signals during stress (one to three minutes), stimulation (two minutes), and post-stimulation (two to eight minutes) were used. For each interval, the ECG signal was inspected visually to avoid ectopic, noisy beats and arrhythmias. Here for HRV analysis, we used a MATLAB-based open source HRV toolbox that was previously validated with a variety of HRV measurement techniques and platforms to calculate LF HRV, HF HRV, LF/HF HRV, SD1, SD2, SD1/SD2 [194].

Electrodermal Activity Measures: Electrodermal activity parameters extracted were SCL, SCL slope, SCR, f_{NSSCR}, L_{SCR} [92]. For SCL, the DC level of EDA signal was extracted and the mean, minimum, maximum, standard deviation, slope of the first order polynomial fit (SCL slope), and area under curve properties were derived. SCR was analyzed in a similar manner to SCL. The peaks in SCR were located by thresholding, and the number of peaks per interval was computed to calculate f_{NSSCR}, excluding the first peak in the signal which corresponds to a specific event (i.e. stress start instance). For L_{SCR}, the latency from the start of the interval to the first peak appearance was calculated. The determination of the minimum peak amplitude was required to define the response occurrence. Although a

minimum of 0.05μS is common with hand scoring of SCR responses, this threshold is largely task- and subject-specific, and can be as low as 0.01μS [209]. We determined the minimum peak amplitude to be two times the rest SCR mean amplitude for each participant, resulting in a mean of 0.06 ± 0.03μs for this study.

4.2.8 Blood Biomarker Assays

We performed two analyses on blood biomarkers: 1. Serum cytokine analyses for all timepoints, 2. PACAP analyses on select timepoints. PACAP analyses were kept exploratory due to budget constraints. For serum cytokines, we performed multiplex assays to measure IL-1ß, IL-2, IL-6, TNF-α, and IFN-γ (Meso Scale Discovery, Rockville, MD). For PACAP, competitive enzyme-linked immunosorbent assay (ELISA) kits were used (LS Bio, Seattle, Washington). Intra-assay coefficient of variation (CV) was 9.89%, and limit of detection (LOD, sensitivity) was 0.288 pg/ml. All experimental operations were in accordance with standard protocols. R2s of the standard curves for each plate were greater than 0.999.

4.2.9 Self-Reported Mood and Distress Scales

Throughout the protocol, visual analog scores (VISAN) representing current mood in five dimensions (nervousness, anxiety, fear, anger, highness) and subjective units of distress (SUDS) were obtained during baseline, after each six neutral recordings, and after each six traumatic stress recordings followed by stimulation. The SUDS is a measure of subjective distress widely used in cognitive behavioral therapy [292]. The SUDS are VISAN are used to assess the level of stress attained in the stress challenges, to verify that the procedure is stressful for the subjects.

4.3 Acute Physiological Biomarkers of tcVNS: Non-PTSD Traumatized Population

We first examined the physiological responses to stress in non-PTSD individuals by processing data from wearable sensing modalities [293]. This sample (collected between May 2017 and October 2018) included 24 participants including 12 females. Ref. [293] presents the CONSORT diagram, and provides demographic data for this sample. Mean age of the participants was 31 (± 9 SD). The active group and sham group participants were similar in age and sex. The active group participants (n = 12) had a mean age of 29 (± 7 SD) and included five females; sham group participants (n = 12) had a mean aged of 32 (± 11 SD), with seven females. In this sample, four (17%) met criteria for past depression, one (4%) for past PTSD, two (8%) for generalized anxiety disorder, one (4%) for past panic disorder, two (8%) for past alcohol abuse or dependence, and one (4%) for a past history of history of drug abuse or dependence. The tcVNS amplitude levels participants received were 18V (± 4.8 SD) for active tcVNS, and 12.6V (± 2.8 SD) for sham stimulus.

4.3.1 Statistical Analysis

We compared participant characteristics between active and sham group using student t-tests (for normal continuous variables), Wilcoxon rank-sum tests (for non-normal continuous variables), and chi-squared tests (for categorical variables), as shown in Table S2. To understand the relative changes in the physiological parameters, data were separated into intervals reflecting the baseline of the corresponding day, stress, stimulation (active or sham), and post stimulation. Absolute and percent changes from the baseline state for each interval were computed and compared between-group differences across the intervals. For physiological parameter intervals (except HRV), data from one minute of baseline rest,

first 30 seconds of stress, last minute of stimulation, and one minute from post-stimulation (three minutes after the stimulation stops) were used. For speech and mental arithmetic tasks which corrupt the respiration waveform due to vocalization, the respiration beats just before the subjects start speaking were extracted as respiratory data during these stressors. These intervals correspond to the end of speech preparation (just before the subject starts speaking after two-minute preparation), and the interval just after the subjects heard the first mental arithmetic question (before answering). For non-continuous BP analyses, similarly SBP, DBP, PP values measured during baseline, stress, stimulation, and post-stimulation were used. Longer intervals for HRV measures were used to comply with the standards. The extracted parameters were evaluated with respect to the corresponding baseline values for each day, either as a ratio with baseline (percent changes) or subtraction from the baseline (absolute changes), for each interval. HRV indices were also evaluated as raw values for each interval. Data in bar plots were represented as mean ± 95% CI plotted from the raw unadjusted values. To evaluate if device type (active vs. sham) was associated with changes in parameters from the baseline value, we used mixed models with repeated measures that included random effect for each participant using unstructured correlation matrix (i.e., multiple traumatic scripts from the first day, two stimulations without acute stress on the first day, two stimulations without acute stress after a 90-minute break on the second and third days, two stimulations followed by two public speech or two mental

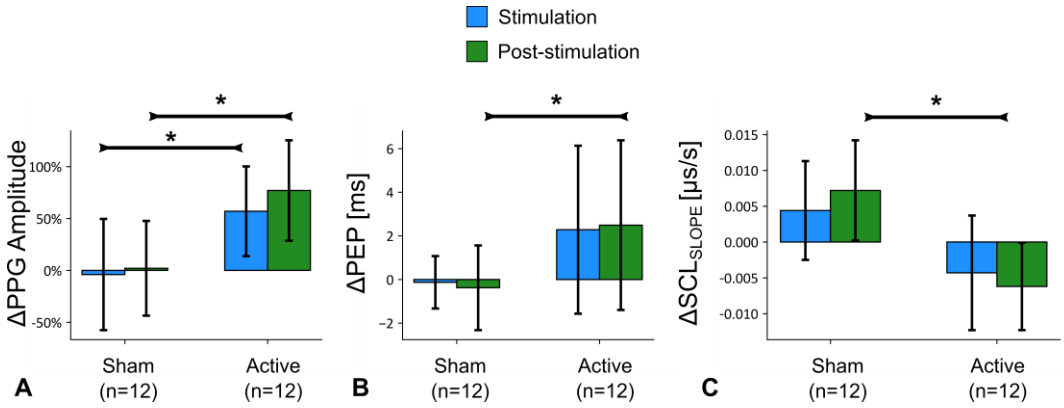

Figure 4-3. Results from physiological signal analyses for stimulation without acute stress from the second and third protocol days. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. a) Active tcVNS group experienced an increase in PPG amplitude during stimulation ($p = 0.049$) and post-stimulation ($p = 0.021$) compared to the sham group. b) Active tcVNS group experienced an increase in pre-ejection period during the post-stimulation interval ($p = 0.035$) compared to the sham group. c) Active tcVNS group experienced a decrease in SCL slope during the post-stimulation interval ($p = 0.014$) compared to the sham group.

arithmetic tasks), and adjusted for age in the models. In a sensitivity analysis, we also tested the significance of the interaction between device type and time variable. Statistical analyses on both percent and absolute changes were carried out in all the models. The beta coefficients (ß) from the mixed models indicate the adjusted average percent or absolute differences in the changes of parameters from the corresponding rest values, comparing active vs. sham device types. ß were reported along with 95% CI and P-values in results and figure captions. A two-sided $p < 0.05$ denoted statistical significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and MATLAB (R2017b, Natick, MA).

4.3.2 Results

tcVNS has a similar effect on SNS activity both in the presence and absence of stress.

To understand the physiological changes induced only by active or sham stimulation, the protocol included two stimulation administrations in the absence of traumatic scripts or mental stress (mental arithmetic and public speech) tasks, after a 90-minute break from the mental stress protocol on the second and third days (Figure 4.3 shows the data from the unadjusted raw changes from the baseline state during stimulation and post-stimulation intervals, results were expressed as mean values, 95% confidence intervals, p-values obtained after adjustments). Stimulation without stress tasks resulted in differences in physiological biomarkers associated with sympathetic tone: PPG amplitude (Figure 4.3a, measurement of peripheral vasoconstriction, inversely related to peripheral sympathetic activity) increased (indicating relative vasodilation and decreased sympathetic activity) during stimulation by 78.6% (95% CI, 0.5-156.7%, p = 0.049), and following stimulation by 95% (15.7-174.2%, p = 0.021) after adjustments in the active tcVNS group relative to the sham group. PEP (Figure 4.3b, inversely related to cardiac sympathetic activity) increased following stimulation by 3.3ms (0.2-6.3ms, p = 0.035) after adjustments in the active group compared to the sham group, indicating a decrease in cardiac contractility and sympathetic activity. SCL slope (Figure 4.3c, related to sympathetic activity) decreased during post-stimulation by -0.013μS/s (-0.024 - -0.003μS/s, p = 0.014) after adjustments in the active tcVNS group relative to the sham group.

tcVNS modulates autonomic tone following exposure to personalized traumatic scripts

Stimulation following exposure to personalized traumatic scripts revealed marked changes in autonomic reactivity between the active and sham groups. Figure 4.4a-c illustrates changes in physiological parameters from the baseline state for the three intervals: traumatic stress, stimulation, and post-stimulation, data shown from unadjusted raw values.

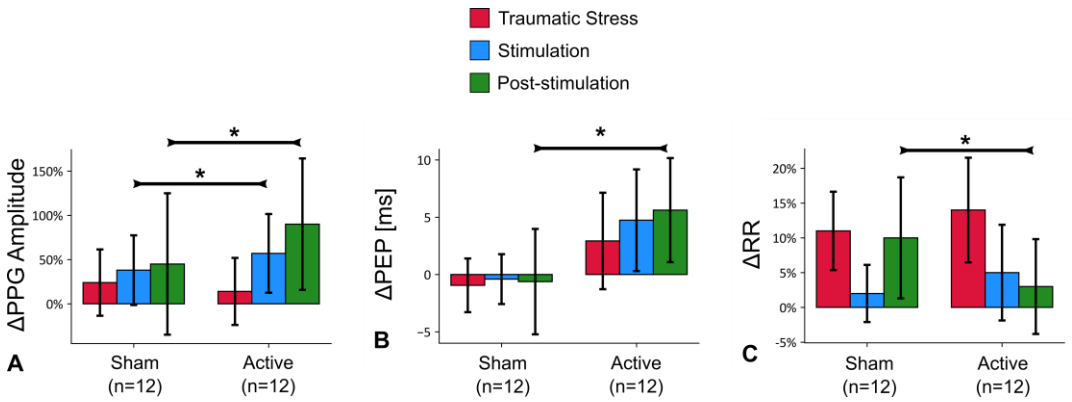

Figure 4-4. Results from physiological signal analyses for stimulation following traumatic stress. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. a) The active tcVNS group experienced a greater increase compared to sham in PPG amplitude during stimulation ($p = 0.036$) and post-stimulation ($p = 0.044$). b) The active tcVNS group experienced an increase in pre-ejection period during post-stimulation ($p = 0.003$) compared to sham. c) Sham group experienced increase in respiratory rate (RR) during post-stimulation ($p = 0.002$).

There were no significant differences in peripheral vasoconstriction measured by PPG amplitude during traumatic scripts between groups. There was an increase in PPG amplitude (indicating relative vasodilation and decreased peripheral sympathetic activity) during stimulation delivered immediately at the termination of traumatic scripts which persisted after the end of stimulation in the active versus the sham group. PPG amplitude was 43.7% higher (3.1%-84.3%, $p = 0.036$, Figure 4.4a) during active versus sham stimulation and 47.9% higher (1.4%-94.5%, $p = 0.044$) in the post-stimulation interval after adjustments. As for PEP, there were no significant differences in PEP during traumatic scripts and during stimulation between groups. In the post-stimulation interval, an increase in PEP (indicating decreased cardiac sympathetic activity) was observed in the active versus sham group with an adjusted difference of 4.2ms (1.6-6.8ms, $p = 0.003$, Figure

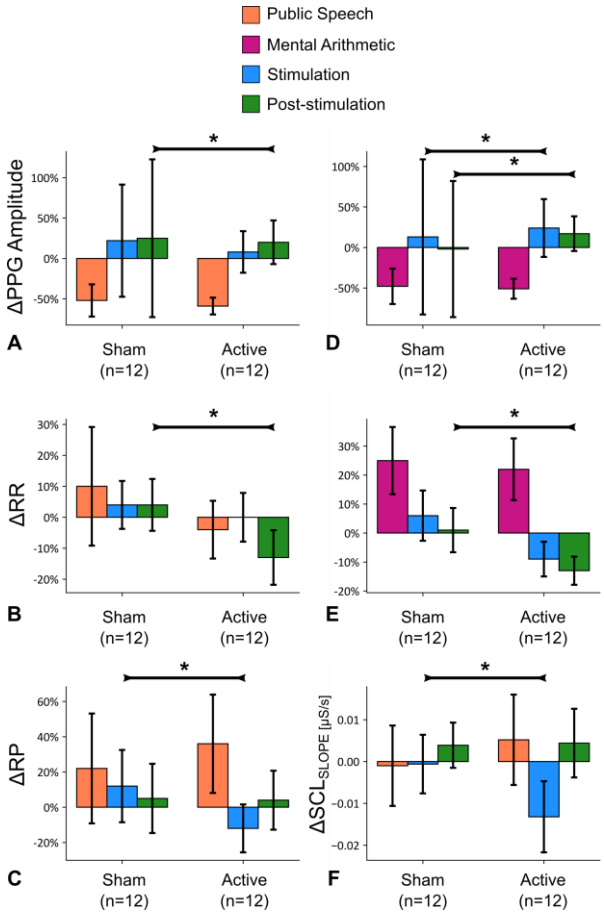

Figure 4-5. Results from physiological signal analyses for stimulation following two types of mental stress, public speech and mental arithmetic. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. a) Increase in PPG amplitude for active group during post-stimulation ($p = 0.009$). b) Decrease in RR for active group during post-stimulation ($p = 0.017$). c) Decrease in respiration RP for active group during stimulation ($p = 0.028$). d) Similar to (a), active group shows a consistent recovery in PPG amplitude during stimulation ($p = 0.005$) and post-stimulation ($p = 0.001$). e) Decrease in RR during post-stimulation for active group ($p = 0.007$). f) Decrease in SCL slope for speech task during stimulation for active group ($p = 0.027$).

4.4b). RR was similar between tcVNS and sham groups during traumatic scripts and stimulation, with an adjusted decrease in the active group relative to sham of -9% (-14.3%--3.7%, p = 0.002, Figure 4.4c) during post-stimulation indicating a release of parasympathetic activity.

Effects of tcVNS on PPG amplitude and respiration rate following mental stress

There were no statistically significant differences during the public speech task between the active and sham groups in PPG amplitude, RR, RP, SCL slope (Figure 4.5a-c, f). PPG amplitude increased during post-stimulation in the active group compared to sham by 61.3% (17.3%-105.3%, p = 0.009, Figure 4.5a) after adjustments. RR decreased in the post-stimulation in active versus sham by an adjusted difference of -11.3% (-20.3%- -2.3%, p = 0.017, Figure 4.5b). RP decreased during stimulation in active versus sham by -25.4% (-47.9%- -3%, p = 0.028, Figure 4.5c) after adjustments. Lastly, SCL slope decreased during stimulation in active versus sham by -0.014μS/s (-0.026- -0.001μS/s, p = 0.027, Figure 4.5f) after adjustments.

Similar to public speech, there were no difference between active and sham groups during the mental arithmetic stress task in PPG amplitude or RR. Active stimulation relative to sham resulted in an adjusted increase in PPG amplitude of 95.8% (32.3%-159.2%, p =0.005), with a post-stimulation adjusted increase of 70.4% (30.8%-110%, p = 0.001) (Figure 4.5d). Following active tcVNS there was a decrease in RR of -14.6% (-24.8%- -4.3%, p = 0.007, Figure 4.5e) after adjustments. Increased PPG following mental stress tasks and tcVNS indicates decreased peripheral sympathetic activity while decreased RR suggests a decrease in parasympathetic withdrawal. As for the two administrations without acute stress on the first day, PEP in active group compared to sham increased by 7.2ms (p = 0.027) after adjustments following stimulation. There were no other marked differences in HR, SBP, DBP, PP, RW, HF HRV, LF HRV, LF/HF HRV, SD1, SD2, SD1/SD2, PAT,

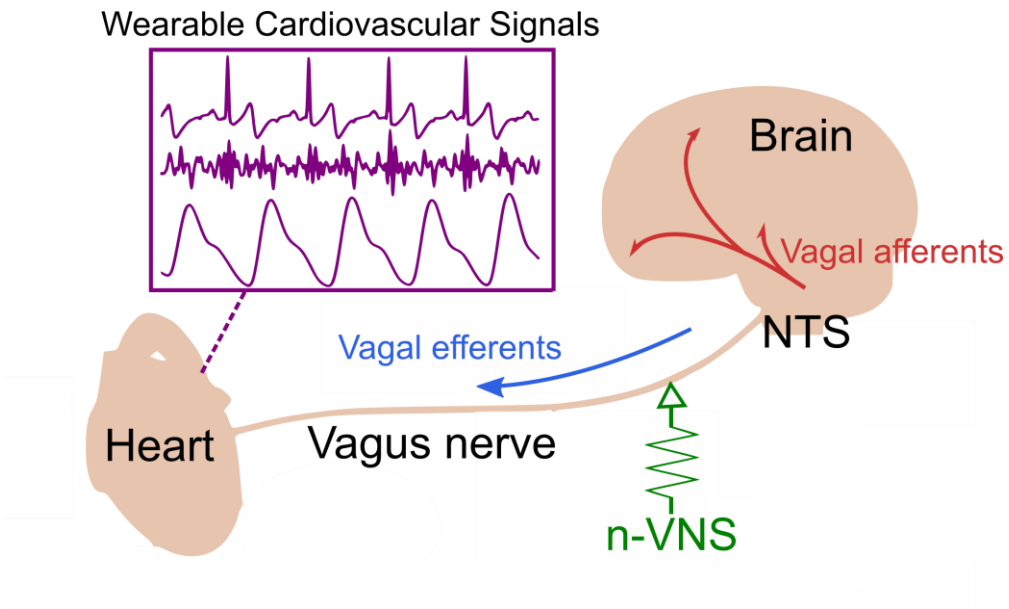

Figure 4-6. Simplified representation of noninvasive VNS mechanism of action. The understanding of noninvasive VNS kinetics on noninvasively obtained physiological parameters may enable optimization of noninvasive VNS delivery in unsupervised settings. NTS: nucleus tractus solitarus.

other parameters related to electrodermal activity, such as SCL, f_{NSSCR}, and L_{SCR} that could distinguish active tcVNS and sham stimulation.

4.3.3 Stimulation Onset Investigation

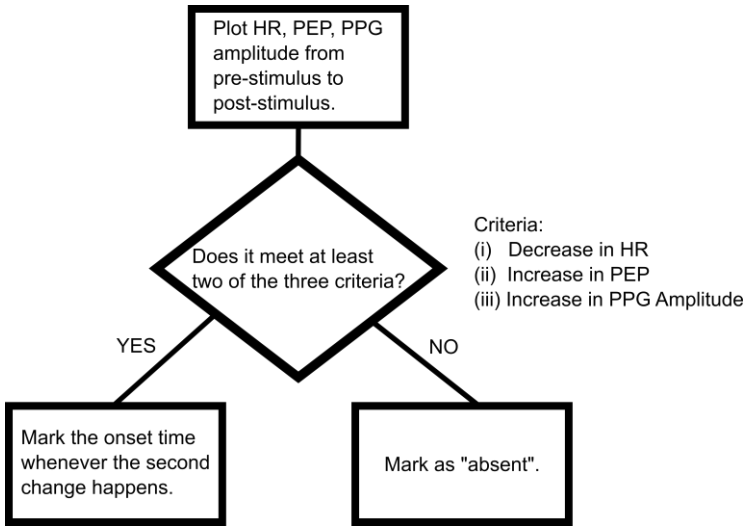

Figure 4-7. Annotation diagram. The smoothed instantaneous biomarkers (HR, PEP, PPG amplitude) were plotted from pre-stimulus to post-stimulus. If at least two of the three mentioned changes in the biomarkers occurred, the onset time was marked at the onset of the second change. If no eligible change was observed, the annotation was marked as “absent”.

tcVNS devices have the potential for widespread applicability in improving the quality of life, however, questions regarding response time, “dosage,” or optimal treatment paradigms remain open. We next investigated the latency of stimulation as seen in the physiological responses from 233 administrations on 24 human participants, with and without immediately preceding acute traumatic stress [294]. Determining the latency from tcVNS initiation to downstream physiological effects (Figure 4.6) serves as a step toward closing the loop with higher temporal resolution for personalized neuromodulation.

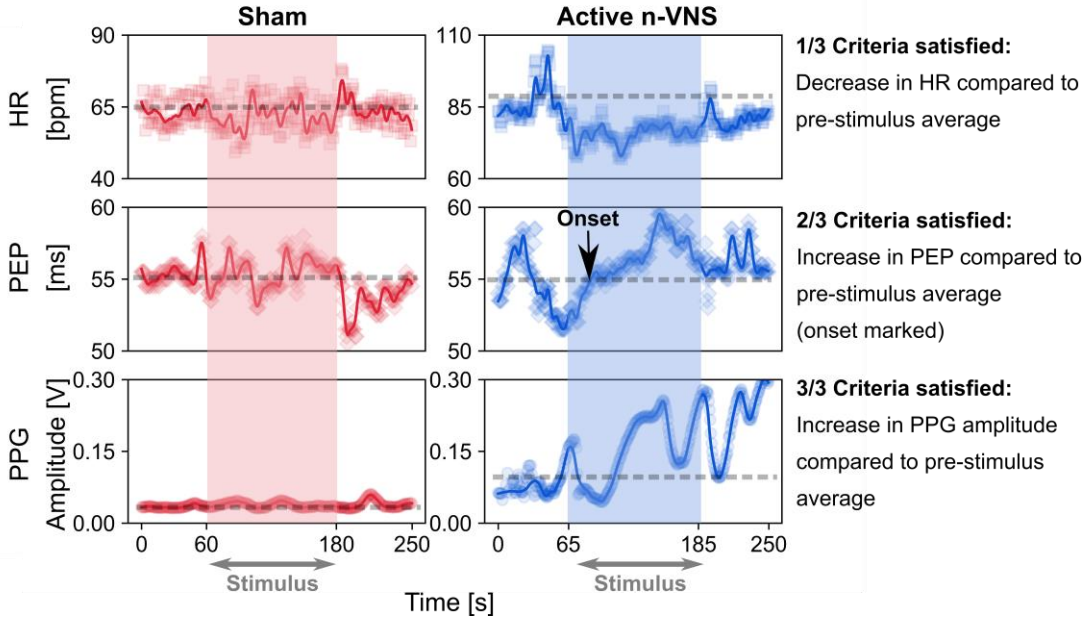

Figure 4-8. Continuous physiological parameters showing tcVNS without traumatic stress, for one participant undergoing sham (left) and one participant undergoing active tcVNS stimulus (right). Markers represent the extracted data, lines represent the smoothed data. Shaded regions represent stimulus delivery. Dashed lines show the averages of the measures from pre-stimulus.

Based on the physiological parameter results, HR, PEP, and PPG amplitude were considered as the most consistent real time biomarkers of stimulation. The anticipated changes following stimulation compared to the period before stimulation were: i) decrease in HR (i.e., decrease in sympathetic tone or increase in parasympathetic tone), ii) increase in PEP (i.e., decrease in cardiac contractility and cardiac sympathetic activity), and iii) increase in PPG amplitude (i.e., decrease in peripheral sympathetic activity). As multiple sensing modalities were used, occasional noisy measurements existed. Therefore, observing the occurrence of all three of these changes following each tcVNS administration was not expected. Thus, as indicated in in Figure 4.7, we located the first datapoint that satisfied two of the three aforementioned criteria and marked this time point as the onset

time. These criteria were established and agreed upon prior to any manual labeling to ensure guidelines were not contorted to match desired results *a posteriori*. To find the corresponding onset of action, we subtracted the tcVNS start time from the labeled onset time. If only one of the three signals experienced noticeable change, the onset of action was marked as "absent." Likewise, if no changes at all were observed or if all changes were relatively insignificant compared to noise and normal variation, the corresponding onset of action was marked as "absent." To allow for independent sampling, we averaged the counts of absent onsets for each participant. Additionally, all annotations were independently

Table 4-1. Onset of action and absent onset counts. Values represent mean ± SD.

tcVNS administrations	**Onset of Action [seconds]**
tcVNS with traumatic stress (n = 72 administrations)	16 ± 9
tcVNS without stress (n = 48 administrations)	18 ± 7
Device groups (p = 0.006)	**Absent Onsets [counts per subject]**
Active tcVNS (n = 12 subjects)	2 ± 2
Sham (n = 12 subjects)	5 ± 2

performed by the three researchers to later assess the inter-annotator agreements for validation purposes. These were calculated as follows: pairwise agreements between the annotators were calculated in seconds (absolute difference between each onset time annotation) and in counts (agreement percentage for absent onsets). The final agreement results reported are the overall average absolute difference in annotated onset times and the average percent agreement in absent onsets.

The continuous physiological parameters from two representative participants in the presence of traumatic stress are shown in Figure 4.8 one participant undergoing sham and

the other undergoing active tcVNS. The pre-stimulus values for each of the biomarkers are shown with dashed lines to give a reference for the predicted deviations. Table 4.1 lists the onset of action for active tcVNS for both with and without traumatic stress, as well as the absent onset counts comparing the active and sham device groups. Based on the physiological biomarkers used, the effects of tcVNS were observed 18 ± 7 seconds from the start of tcVNS without stress. When tcVNS was applied after traumatic stress (six traumatic stress scripts followed by six tcVNS per participant, n = 12 participants), effects were observed on the biomarkers in a similar latency, resulting in 16 ± 9 seconds. As for the absent onset counts (tcVNS administrations that had not met the criteria), there is a significant difference between the device groups: there were 5 ± 2 absent onsets per participant for the sham group, significantly higher than the active group's 2 ± 2 absent onset counts ($p = 0.006$). There were 24 and 65 absent onset counts for 120 active tcVNS and 113 sham administrations, respectively. The overall average of inter-annotator agreements resulted in a 4 ± 1 seconds difference between the labeled onset times and a 90 ± 5.5% agreement in absent onset counts.

4.3.4 Discussion and Conclusion

We demonstrated the feasibility and utility of quantification of cardiovascular and peripheral autonomic nervous system function using wearable sensing devices in conjunction with administration of tcVNS and a sham control and stressful tasks. tcVNS minimized sympathetic activation and/or withdrawal of parasympathetic tone following exposure to stress based on a range of physiological parameters. This was observed for different kinds of stressors, including exposure to recordings of personalized traumatic memory scripts, and "neutral" or "mental stress" tasks including mental arithmetic and

public speech stress tasks. The findings suggest that wearable sensing devices could be used as real-time non-invasive physiological biomarkers of tcVNS to predict treatment efficacy and/or provide empirical evidence of proper tcVNS administration.

tcVNS shows effects in multiple physiological biomarkers

Active tcVNS compared to the sham group resulted in a decrease in peripheral and cardiac sympathetic activation for tcVNS alone as measured by increased PPG amplitude, increased PEP, and decreased SCL slope. There was also a reduction in peripheral sympathetic activation with tcVNS applied after both traumatic script and mental stress tasks as measured by increased PPG amplitude, and decreased cardiac sympathetic activation after traumatic scripts (but not mental stress) based on increased PEP. In a complementary manner, tcVNS resulted in reduced parasympathetic withdrawal after both traumatic scripts and mental stress tasks based on reduced RR. tcVNS also decreased SCL slope when followed by public speech task (but not arithmetic or traumatic stress). The use of various stressors revealed task-specific changes in autonomic nervous system activity: while an increase in PEP was observed for tcVNS when applied following a traumatic stressor, an increase in PEP was not observed upon stimulation for mental stressors. Similarly, reduction in SCL slope was observed for stimulation without acute stress and stimulation following public speech only. On the other hand, tcVNS resulted in increases in PPG amplitude and decreases in RR when applied after both traumatic script and mental stress tasks. Thus, there is not a single biomarker of tcVNS, rather its efficacy could be revealed from signals that are related to different pathways of autonomic reactivity to different types of stressors.

Changes in PPG-amplitude versus lack of changes in blood pressure

Increased PPG amplitude with tcVNS was one of the most consistent results across the various stressful tasks in this study. However, a myriad of factors is involved in affecting the amplitude of PPG signals, and thus associating changes in PPG amplitude changes with a particular underlying physiological origin is not straightforward. To the first order, the two main factors influencing PPG amplitude are pulse pressure and arterial compliance [295]. Thus, it is important to note that in this study we did not observe differences between the active tcVNS and sham groups in systolic, diastolic blood pressure, and pulse pressure for any of the intervals – this indicates that the changes in PPG amplitude that were significant, and quite substantial, were linked to local changes in arterial tone associated with vasoconstriction and vasodilation. Accordingly, the effects of tcVNS on PPG amplitude may be attributed to sympathetic regulation of vascular tone.

No effects of tcVNS on HRV

The current study did not find significant differences between the active tcVNS and sham groups in ECG-based measurements of heart rate variability (HRV) either for short intervals during and after stimulation, or for whole day measurements.

Effects of tcVNS on HR and electrodermal activity (EDA)

In this sample, HR measurements are not significantly different between active and sham groups for any of the intervals analyzed. While this non-PTSD sample did not reach to statistical significance, a larger sample (taken later for increasing the blood data sample) resulted in decrease in HR. Our findings are also consistent with studies observing no HRV

or EDA changes following auricular VNS in combination with subjectively measured fear and anxiety [296-298]. A recent study on the effects of tcVNS with a noxious stressor (thermal stimuli) reported difference in EDA-related changes in active and sham group, specifically in SCL slope and L_{SCR} [180]. Our analysis has shown difference for SCL slope, but not for latency of SCR.

Use of seismocardiography (SCG) in the assessment of effects of tcVNS on peripheral autonomic function

The current study found PEP, a measure of sympathetic activity, to be useful in the assessment of the effects of tcVNS on autonomic function. PEP has been studied as a measure of cardiac sympathetic activity (or cardiac contractility), along with comparisons with HRV, EDA, and plasma catecholamines [202, 277, 279, 299-302]. We observed in our study that tcVNS administration creates electrical stimulation artifacts on ICG signal as the stimulation bandwidth and ICG signal bandwidth coincide with each other. Supplementary Figure S4 in Ref. [293] shows SCG versus ICG during tcVNS and noise hiding the fiducial point to extract the PEP (known as B-point, representing the opening of aortic valve) [202]. SCG is a viable option to calculate PEP in clinical studies that use tcVNS as it is a mechanical signal reflecting the chest-wall vibrations of the heart, hence the electrical stimulation does not affect the waveform shape. Beat-by-beat analysis during the treatment is possible with SCG-derived PEP. SCG also does not require electrodes, unlike ICG.

Limitations

The following limitations should be noted for this study. Prior animal studies initiated direct VNS or sham before the initiation of the fear-related stimulus [145, 303]. Other studies in human subjects initiated taVNS or sham before or during the stimuli [172, 296, 298]. Therefore, stimulation prior to stress and concurrently with stress appear to improve the pathological response based on previous studies. This study employs a reactive acute treatment approach as stimulation administrations were applied right after the stressors ended. Subjects were instructed, however, to form an image from the traumatic scripts in their mind and hold it, and stimulation was applied immediately at the end of the script. Our prior experience with traumatized subjects including those with PTSD demonstrated that upsettedness typically continues after the termination of the script, stress- or fear-related task [51, 304]. Therefore, we believe that the stimulation was applied at the peak of the behavioral effects of the task. Future studies should investigate the effects of preemptive versus reactive stimulation in the context of traumatic stress.

Due to the clinical nature of this study, the target engagement of the cervical vagus nerve could not be validated directly. This study relies on previous literature that reported the ability to reach the vagal afferents using tcVNS [174, 176]. We replicated the stimulation application reported in [174] throughout the protocol, by locating the carotid artery as an anatomical reference. Although variation exists regarding the location and topographical anatomy of the cervical vagus nerve, a recent cadaveric study reports that cervical vagus nerve can be visualized in a 35×35 mm distance lateral of the laryngeal eminence and posterior to the skin of the neck, which typically falls under the area the electrodes are placed [305].

A natural restriction of this study is the possibility of therapeutic effects from traumatic exposure (traumatic stress rehearsal) [306]. Figure 4.4 combines data from all six traumatic stress scripts per subject, showing increases only in RR during traumatic stress. To show how the subjects respond to traumatic stress initially, we also analyzed only the first traumatic script responses for the primary outcome variables, excluding all other repetitions, for each subject, as supplementary information in Ref [293]. It is seen that HR and RR increase, PPG amplitude decreases during traumatic stress. The lower stress reactivity in Figure 4.4 might be due to the therapeutic effects of the repetitions as the data were merged from six traumatic scripts per subject (repetition numbers were included in our statistical analyses). Nevertheless, as our study focuses on tcVNS effects on stress, our main consideration was whether the active and sham groups received comparable amounts of stress. We did not observe significant differences in stress responses. Therefore, although repeated exposure might change stress reactivity over the time, the reactivity remained similar between the active and sham groups, which facilitated comparison of the effects of active and sham stimulation.

The functional relevance of the PPG amplitude results could be attributed to changes in total peripheral resistance (TPR) or pulse pressure (PP), however there is no direct linear correlation to either. The PPG signal is an optical measurement, the amplitude of which is determined by the Modified Beer-Lambert Law [295]. PPG amplitude reflects the expansion and contraction of the vessel diameters in the region (index finger) being illuminated by the light source. This expansion and contraction of vessel diameter is proportional to both PP and arterial compliance. Compliance is the change in a vessel's volume for a given change in PP. Thus, while directional relationships between PPG

amplitude changes and TPR can be quite informative, the attribution of a given change in amplitude to a particular change in TPR is complex. Nevertheless, the study did not find remarkable differences in non-continuous BP measures (SBP, DBP, PP), or PAT. This is an interesting result considering the relationship of PPG and BP waveforms [200, 206]. PPG measurements (hence the extracted PPG amplitude and PAT) were continuous, and thus beat-by-beat assessment was feasible —a desirable measurement for the acute characteristics of this study. BP measurements were taken through a blood pressure cuff, and hence BP changes at beat-by-beat level could not be assessed. Future studies should examine whether continuous BP is affected by tcVNS.

While assessing the mental stress reactivity to tcVNS, it is important to clarify that the active and sham groups reacted similarly to mental stressors, which permits the comparison of stress response upon stimulation between the groups. The public speech task is a version of Trier Social Stress Test [307]. Traumatic stress protocol, public speech, and mental arithmetic tasks have been verified multiple times to induce significant psychobiological and cardiovascular responses on human subjects [190, 308-310]. In this study, similar responsivity between the groups were seen in the measures analyzed during the stressors. The groups showed no significant difference during stress intervals in any of the cardiovascular, peripheral, electrodermal activity measures.

tcVNS affects physiological signals in >10 seconds

Our findings from 233 administrations suggest pertinent timing considerations germane to the design of effective clinical studies involving physiological effects of tcVNS. Wearable cardiovascular and peripheral biomarkers —HR, PEP, and PPG amplitude— were

modulated in 18 ± 7 seconds for tcVNS without stress and in 16 ± 9 seconds for tcVNS following traumatic stress with significant difference in the absent onset counts between active and sham groups. The utility of this work is to eliminate ambiguity of the physiological outcomes for tcVNS studies. The findings of this study have applications in clinical studies that use tcVNS in tandem with or without stress; as well as in the design of wearable systems that combine sensing and stimulation. For instance, the timing of the stimulation appears to be important in the outcomes of multiple studies that test the differences in cognitive functioning, memory functioning, psychomotor functioning, or executive functioning with VNS application [124, 149, 153, 311-314]. The outcomes of onset investigation could be used to design effective clinical studies for tcVNS devices. From the wearable sensing standpoint, the results could be instrumental for decision making algorithms, such as the determination of stimulation timing or the effectiveness of the stimulation. The inclusion of the sham group and multi-day protocol presented in this study provides unique, continuous wearable sensing data to consider for physiological outcomes of similar studies.

Conclusion

In summary, our investigation demonstrates that tcVNS has effects on peripheral autonomic function that can be feasibly and reliably measured with wearable sensing devices. Specifically, tcVNS both in isolation and following exposure to stress reduces sympathetic and enhances parasympathetic function, leading to a modulation in autonomic tone. These physiological biomarkers may be useful for long-term monitoring of tcVNS in the home setting to assess adherence and accuracy of neuromodulation treatments and to provide subject-specific dosage recommendations for tcVNS therapy. tcVNS also

minimizes sympathetic activation in response to stress, which suggests that it may have clinical applications to stress-related psychiatric disorders characterized by increased sympathetic activity that is correlated with symptoms of these disorders [282, 283, 315-318]. The fact that tcVNS reduces or blocks sympathetic arousal associated with exposure to personalized traumatic scripts suggests a clinical application to patients with PTSD in the context of modulation of indelible traumatic memories and possible enhancement of neuroplasticity and/or facilitating extinction of conditioned responses to reminders, which were previously studied in preclinical literature through direct VNS with implantable devices [145, 303, 319]. Although not assessed in the current study, emerging findings of the beneficial effects of direct VNS on cognition and memory suggest other possible benefits of tcVNS for patients with stress-related psychiatric disorders [124]. tcVNS could have a potentially broad impact in the domains of human performance and mood improvement, and wearable sensing devices can be used to quantify the stimulation. This could be applicable to other clinical and neuroscience research environments and in general wearable bioelectronic medicine, for patients with or without psychiatric disorders.

4.4 tcVNS Reduces Sympathetic Responses to Stress in PTSD

With the same experimental protocol, we analyzed data from patients with PTSD (n = 25). Preprint [320] (under review as of 7/4/2020) details the CONSORT requirements and details of the study sample used in this analysis.

4.4.1 Patients with PTSD

The mean age was 35 (± 13 SD) with 19 females. The active group participants (n = 13) had a mean age of 33 (± 12 SD) and included 12 females; sham group participants (n = 12)

had a mean age of 38 (± 13 SD), with seven females. Upon SCID evaluation, 13 (52%) met criteria for major depressive disorder (MDD, 6 current, 7 past), eight (32%) for generalized anxiety disorder, four (16%) for panic disorder, two (8%) for social phobia, two (8%) for current obsessive compulsive disorder, one (4%) for agoraphobia without panic disorder, one (4%) for body dysmorphia, three (12%) for past alcohol abuse or dependence, and one (4%) for a past substance induced anxiety disorder. In terms of stimulation amplitude, active group received 20.3V (± 7.5 SD), and sham group received 13.6V (± 1.4 SD) averaged across all uses over three days.

4.4.2 Signal Processing, Parameter Extraction, and Statistical Analysis

We carried out the same signal processing and statistical analysis for PTSD sample to allow for comparison of findings with non-PTSD sample as a future investigation. Results were reported as ß values, 95% CI, effect sizes, and p-values.

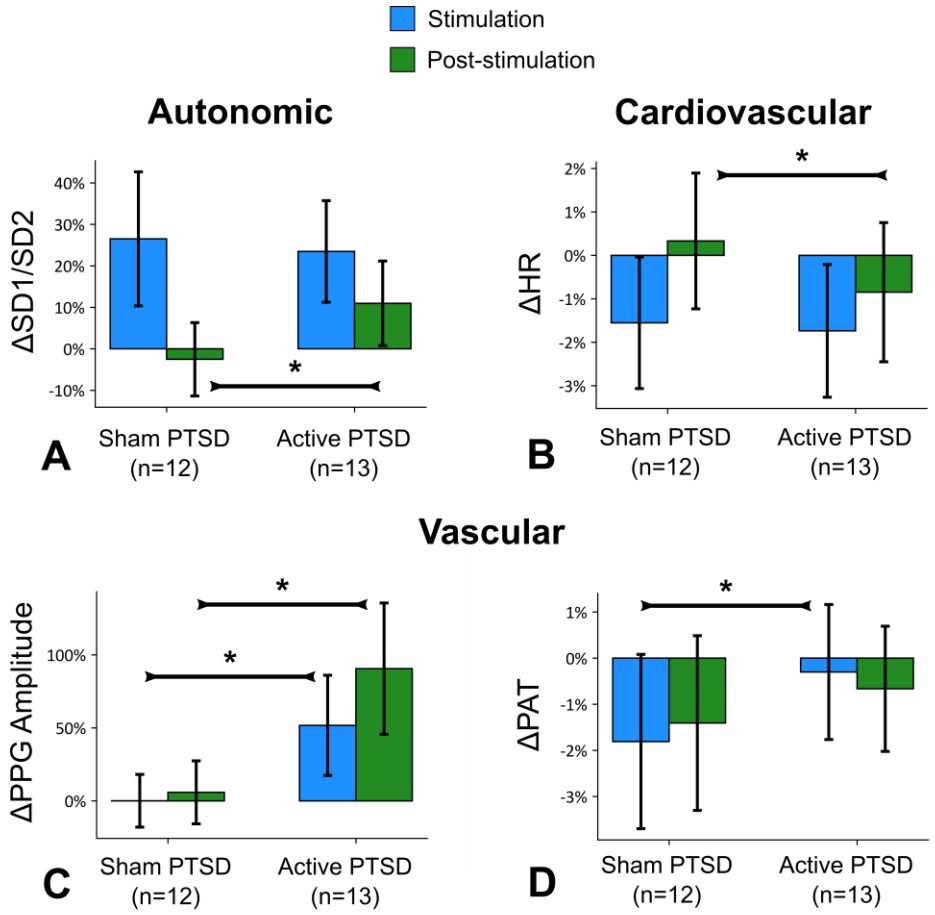

Figure 4-9. tcVNS without acute stress: Results for stimulation without acute stress, merged from all days. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. ß coefficients, adjusted CI, effect sizes (d), and p-values were reported in ß (± CI, d, p) format. Active tcVNS group experienced the following relative to sham after adjustments: a) The ratio of short-term variability to long-term variability (SD1/SD2) increased following stimulation by 14.1% (±11.6%, d=0.43, p = 0.019). b) HR decreased following stimulation by 2.7% (±2.0%, d = 0.21, p = 0.009). c) PPG amplitude increased during stimulation by 43.4% (±43.4%, d = 0.53, p = 0.049) and following stimulation by 73.1% (±63.2%, d = 0.67, p = 0.025). d) PAT increased during stimulation by 2.5% (±2.2%, d = 0.26, p = 0.026).

4.4.3 Results

tcVNS consistently decreases SNS in absence of stress over multiple days.

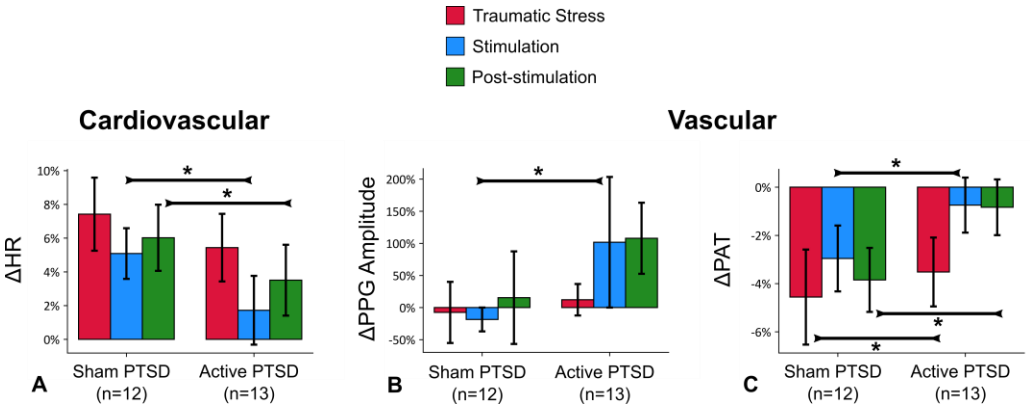

Figure 4-10. tcVNS after traumatic stress: Outcomes for stimulation following traumatic stress (all six scripts). Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates p < 0.05. ß coefficients, adjusted CI, effect sizes (d), and p-values were reported in ß (± CI, d, p) format. Active tcVNS group experienced the following relative to sham after traumatic stress after adjustments: a) HR decreased during stimulation by 5.6% (± 3.6%, d = 0.43, p = 0.003), and following stimulation by 3.9% (± 3%, d = 0.29, p = 0.013). PPG amplitude increased during stimulation by 30.8% (± 28.0%, d = 0.41, p = 0.032). c) PAT decreased less during traumatic stress by 9.2% (± 3.0%, d = 0.15, p < 0.0001), stimulation by 2.2% (± 2.2%, d = 0.42, p = 0.045), and following stimulation by 6.2% (± 1.9%, d = 0.57, p < 0.0001).

Figure 4.9 presents the raw values for autonomic (SD1/SD2), cardiovascular (HR), and vascular (PPG Amplitude and PAT) tone. Compared to sham, active tcVNS increased SD1/SD2 (Figure 4.9a, post-stimulation, ß (± CI, d, p) 14.1% (± 11.6%, d=0.43, p = 0.019)), decreased HR (Figure 4.9b, following stimulation, 2.7% (± 2.0%, d=0.21, p = 0.009)), increased PPG amplitude (inversely associated with peripheral sympathetic activity, Figure 4.9c, during stimulation, 43.4% (± 43.4%, d=0.53, p = 0.049); following stimulation 73.1% (± 63.2%, d = 0.7, p = 0.025)), and increased PAT (inversely associated with peripheral sympathetic activity, Figure 4.9d, during stimulation, 2.5% (± 2.2%, d = 0.26, p = 0.026)).

tcVNS reduces sympathetic tone following exposure to personalized traumatic scripts

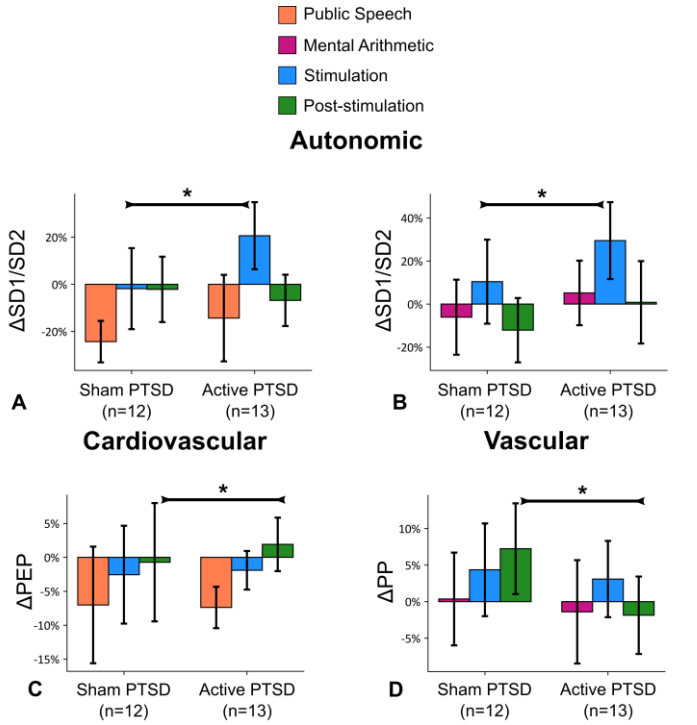

Figure 4-11. tcVNS after mental stress: Outcomes for stimulation following two types of mental stress, public speech and mental arithmetic. Bars represent the unadjusted mean changes from baseline, error bars: 95% CI, values calculated from raw data, * indicates $p < 0.05$. ß coefficients, adjusted CI, effect sizes (d), and p-values were reported in ß (± CI, d, p) format. Active tcVNS group experienced the following relative to sham after adjustments: a) SD1/SD2 increased during stimulation right after speech task by 23.1% (± 21.1%, d = 0.71, p = 0.033). b) Similar to the speech task, SD1/SD2 increased by 41.2% (± 22.5%, d = 0.44, p = 0.001). c) PEP increased following stimulation after speech task by 6.8% (± 5%, d = 0.16, p = 0.009). (D) PP decreased following stimulation after mental arithmetic by 9.6% (± 9.7%, d = 0.68, p = 0.049).

Stimulation following exposure to personalized traumatic scripts resulted in marked changes in autonomic reactivity, similarly to stimulation without stress. Figures 4.10a-c illustrate changes in from the baseline state for the three intervals: traumatic stress, stimulation, and post-stimulation, merged from all six traumatic stressors. Relative to sham, active tcVNS decreased HR (Figure 4.10a, during stimulation, 5.6% (± 3.6%, d = 0.43, p = 0.003); following stimulation, 3.9% (± 3.0%, d = 0.29, p = 0.013)), increased

PPG amplitude (Figure 4.10b, during stimulation, 30.8% (± 28.0%, d = 0.41, p = 0.032)), increased PAT (Figure 4.10c, during combined traumatic stress, 9.2% (± 3.0%, d = 0.15, p < 0.0001); during stimulation, 2.2% (± 2.2%, d = 0.42, p = 0.045); following stimulation, 6.2% (± 1.9%, d = 0.57, p < 0.0001)), indicating attenuation in the elevated autonomic tone due to stress. These effects were not initially observed, as no differences (p > 0.05) were found between active and sham during the first traumatic script.

tcVNS affects cardiac contractility and heart rate variability following mental stress.

Figures 4.11a-d summarize the effects of tcVNS when applied after two different mental stress tasks: public speech and mental arithmetic on the second and third days. SD1/SD2 increased during stimulation right after speech task (Figure 4.11a, inversely related to sympathetic activity, during stimulation, 23.1% (± 21.1%, d = 0.71, p = 0.033)) and after mental arithmetic test (Figure 4.11b, during stimulation, 41.2% (c 22.5%, d = 0.44, p = 0.001)).

Active tcVNS increased PEP (Figure 4.11c) following stimulation compared to sham (6.8% (± 5%, d = 0.16, p = 0.009)), indicating a decrease in cardiac contractility and sympathetic activity. Active tcVNS also decreased PP following stimulation after the mental arithmetic task compared to sham (9.6% (±9.7%, d = 0.68, p = 0.049); Figure 3D), indicating a decrease in vascular reactivity

4.4.4 Discussion and Conclusion

This study showed that tcVNS modulates autonomic, cardiovascular, and vascular measures in PTSD with or without exposure to traumatic and mental stress. The broad

interpretation of the changes due to tcVNS are similar to the sample involving non-PTSD controls (i.e., reduction of sympathetic tone at baseline and blocking sympathetic responses to stress) [293]. Additionally, the effects of tcVNS on vascular measures (i.e., PPG amplitude) persist as markers of autonomic changes with tcVNS independent of disease status.

Active tcVNS decreased sympathetic arousal as measured by autonomic, cardiovascular, and vascular measures across multiple days and types of stressors. Results were seen on the first day after multiple exposures to personalized traumatic script stress, and on the afternoons of the second and third days after exposure to mental stress challenges (public speaking, mental arithmetic) in the morning. PPG amplitude was a persistent biomarker of stimulation regardless of the disease status. We found greater SD1/SD2 response to tcVNS than sham, while the other frequency domain metrics were not affected by tcVNS. While the biological significance of this measure/finding is not clear, it is complemented by auricular VNS studies in which frequency-domain HRV improved [156, 157, 177]. SD1/SD2 is a non-linear Poincaré-based HRV, which is less studied in literature, although another study noted that it is negatively associated with diabetes [321]. Hemodynamic measures (BP, HR) have been studied more, and been mixed throughout tcVNS studies: tcVNS decreased HR in humans [177] and rats, albeit only momentarily [181]. Other studies, however, have not observed any autonomic or cardiovascular changes [182].

tcVNS improved recovery from traumatic stress (reduced HR, increased PAT) and decreased peripheral sympathetic activity (decreased PPG amplitude). The results except PPG amplitude (HR and PAT) differ from the non-PTSD cohort results for traumatic stress. Additionally, no difference in PEP or EDA were noted for the PTSD cohort. With the

merged data (Figure 4.10c), sham group experienced more reactivity to stress. Patients with PTSD fail to habituate to repeated exposure of stress [322, 323]. The therapeutic potential of tcVNS is shown by its effect in decreasing stress reactivity. These results suggest that repeated tcVNS enhances resilience in the face of repeated stress in patients with PTSD.

Active tcVNS improved cardiac contractility recovery following the speech task. PEP is an index of effort-related cardiac activity [324], with greater PEP indicated decreased effort and cardiac sympathetic activation. PEP is responsive to tasks requiring effortful active coping [324], similar to the speech task, potentially indicating tcVNS mitigates effort during the speech task. PEP has been shown previously to respond differently to challenge or threat conditions, specifically resulting in decreases with challenge and minimal changes with threat [325]. In the current study, PEP decreased with the speech task in both groups, and the decrease was mitigated with active tcVNS. The traumatic stress perhaps could be regarded as threat for patients with PTSD. PEP outcomes for speech tasks and (the lack of) PEP outcomes for traumatic stress might be due to the perceptional differences for challenge versus threat [326]. A comparison of previous works in PTSD notes more than double cortisol release with cognitive challenge [327], compared to the cortisol levels with traumatic stress [328]. Although there is no direct statistical comparison between these studies, the magnitude differences in cortisol levels are apparent.

Several aspects of this study might limit the generalization of the results. The active group was female-dominated due to the small sample size. However it is important to note that PTSD is twice more prevalent in females than males [20]. The timing of the stimulation is also relevant to consider in the interpretation of our results. Prior animal and human studies initiated the stimulation before or during the stimuli [145, 172, 296, 297, 303]. In our study,

we stimulated immediately at the end of the exposure to stress in terms of listening to scripts or performing mental stress [50, 51]. Given instructions to hold images of trauma in the mind, and based on our prior work showing that physiological indicators of stress persist after termination of the task, we feel that this timing corresponds to the peak period of stress as experienced by the individual. Our findings on the effects of tcVNS using this protocol on autonomic function, however, are consistent with prior preclinical and clinical studies of VNS using various stimulation timings and protocols [144, 145, 156, 172, 319]. Future studies should compare stimulation before, during, and at the termination of stress protocols.

In summary, using a multimodal sensing approach, we found that tcVNS at rest and paired with various stressors modulates the autonomic nervous system and cardiovascular reactivity. We demonstrated feasibility of use of wearable sensing devices for measurement of novel physiological markers in patients with PTSD. These modalities can be used in a home setting to assess target engagement and treatment efficacy for personalized neuromodulation. Future work should focus on methods to evaluate longitudinal outcomes and parameter determination studies for selective modulation of autonomic tone and to utilize these modalities in assessment of response to neuromodulation treatments in PTSD [155, 329].

4.5 tcVNS Mitigates Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Response to Stress

We also investigated relevant serum cytokines and cellular stress biomarkers. This part of the work was in collaboration with Bradley D. Pearce's research group (Yunshen Jiao and

Allison Hankus) at Rollins School of Public Health, Department of Epidemiology at Emory University. This part of the work is currently under review as a journal paper. Blood samples were processed at Bradley D. Pearce's lab. I evaluated the effect of tcVNS by comparing PACAP concentration across timepoints spanning three days from the analyzed blood data. Due to budget constraints, a limited number of blood draws (baseline and end of each day, timepoints 0, 14, 18, 22 in Figure 4.12) were processed. We also investigated PACAP concentration with baseline psychological assessments.

4.5.1 PACAP Analysis Sample

A total of 36 subjects who completed or at least partly completed the trauma tasks were involved in these analyses. Subject groups were similar in age, body mass index (BMI), race, education level and marital status. Although subjects were randomly assigned to the tcVNS treatment or sham treatment groups, only female PTSD patients received tcVNS treatment. The gender proportion in the other groups was similar. The average age of this population was 30.3 (SD = 9.0) years, and the average BMI was 26.6 (SD = 5.6) kg/m^2. Among all the 36 subjects, 21 (58.3%) were female, 18 (50%) White / Caucasian, 21 (58.3%) had a Bachelor's or higher degree, and 24 (66.7%) had never married.

4.5.2 Statistical Analysis

Since the distribution of PACAP concentration was skewed, log-transformation was applied to PACAP concentrations to achieve normality and better interpretation in all the subsequent analyses. These (log) PACAP data were confirmed to be normal using Shapiro-Wilk test. Initially, we computed partial correlations between (log) PACAP concentration (baseline, maximum, quartiles (lower (Q1, 25th percentile), medium (Q2, 50th percentile),

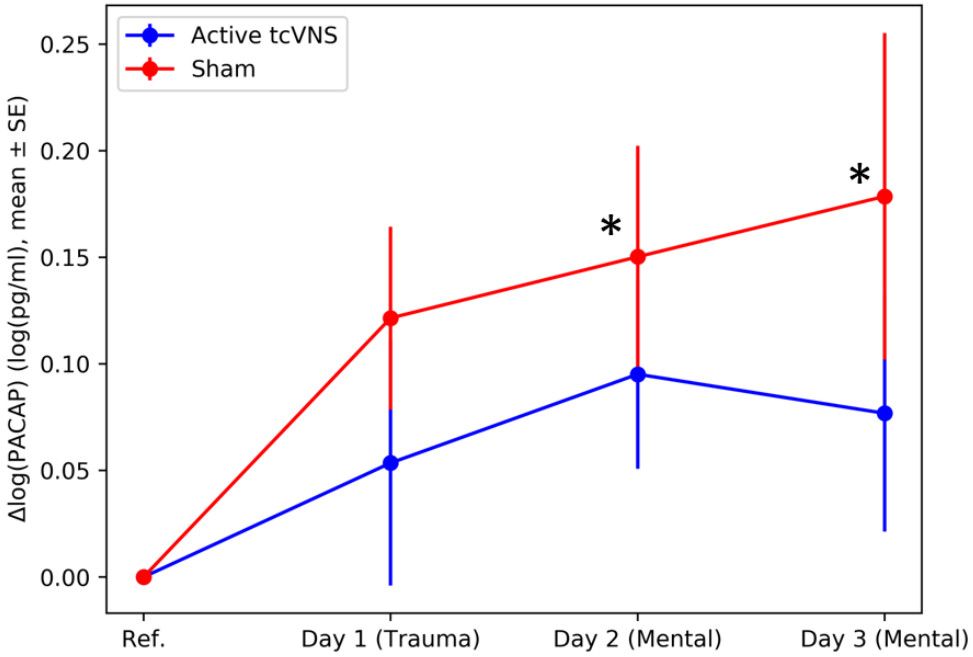

Figure 4-12. Change in PACAP concentration over three days for active tcVNS and sham groups. * indicates $p \leq 0.05$ after post-hoc corrections, error bars are standard error of the mean. Sham group had a marked increase in PACAP, consistently increasing over the course of three days. This elevation was less in active tcVNS group.

upper (Q3, 75th percentile)) for each subject) and psychometric scales, controlling for age, sex, BMI, race, and education and reported correlation coefficients (rho, ρ) and p-values. To assess the trajectory of PACAP concentration over time, we computed the delta (from baseline) at each timepoint. We used a linear mixed model to regress the delta PACAP values on timepoints (0, 14, 18, 22), PTSD status (yes/no), device type (active tcVNS/sham), as well as other covariates (BMI) with subject-specific random intercepts to account for within-subject correlations. When a significant main effect or interaction was present, pairwise post-hoc comparisons with Bonferroni-Holm corrections were used to determine location of differences. For this analysis, an a priori planned comparison

approach yielded the number of tests as five: successive time points (e.g., 14 vs 18) and all numbers different from 0. Effect sizes (Cohen's d) were reported for results, regardless of the significance of p-value, based on group means, standard deviation, and group sample size for device and PTSD status main effects [330]. For post-hoc analyses that include timepoints, repeated measures Cohen's d (d_{rm}) was calculated that includes a correction factor based on the correlation of PACAP concentrations between two timepoints (for example, correlation of delta PACAP between timepoints 0 and 18 and between timepoints 0 and 22 were included as a correction factor) [330]. Missing biomarker data were assumed to be missing completely at random for reasons such as hemolyzed sample or missing baseline data, and all analyses were based on available data. Statistical analyses were done using R (v 3.6.0) and MATLAB R2020a.

4.5.3 Results

First, the association between PACAP baseline concentration and scores of psychological and functional scales were examined. Baseline PACAP concentrations were significantly positively correlated with total PTSD Symptom Score (PTSD-SS) ($\rho = 0.45$, $p = 0.04$) and significantly negatively correlated with Baecke Sports Index ($\rho = -0.46$, $p = 0.05$). The maximum PACAP concentrations (from timepoints 0 to 22 for each subject) were significantly positively correlated with Hamilton Anxiety Scale (HAM-A) ($\rho = 0.43$, $p = 0.05$) and Hamilton Depression Scale (HAM-D) ($\rho = 0.45$, $p = 0.04$). Upper quartile PACAP concentrations (from timepoints 0 to 22 for each subject) were significantly negatively correlated with Baecke Leisure Index ($\rho = -0.53$, $p = 0.03$). None of the other psychological and functional scales were statistically significant with the examined PACAP concentrations.

Figure 4.12 shows the change in PACAP concentration over three days for active tcVNS and sham groups. When examining the PACAP values within the overall sample, the main effect of device type ($p = 0.26$, $d = 0.46$) and PTSD status ($p = 0.13$, $d = 0.12$) along with interactions of device type by time ($p = 0.53$), device type by time by PTSD status ($p = 0.59$), PTSD status by time ($p = 0.48$), and PTSD status by device type ($p = 0.63$) were not significant. However, the main effect of time was statistically significant ($p = 0.008$). Post-hoc comparisons revealed elevated PACAP at timepoints 18 ($p = 0.03$, $d_{rm} = 0.27$) and 22 ($p = 0.03$, $d_{rm} = 0.28$), but not at timepoint 14 ($p = 0.27$, $d_{rm} = 0.18$). No other significant differences were observed at any timepoint for the overall sample ($p > 0.05$).

Although the device type by time interaction was not significant, a subsequent analysis was completed using an isolated dataset with just sham or active across time to better determine the contributions of treatment group to the overall time effect. No significant main effects or interactions were observed for active tcVNS ($p = 0.21$). The active group effect sizes were $d_{rm} = 0.12$, $d_{rm} = 0.21$, and $d_{rm} = 0.18$, for days 1, 2, and 3, respectively. We further examined whether the effect was driven by the sham group. In our isolated analysis, a significant main effect of time was observed in the sham group ($p = 0.04$). Post hoc tests revealed significant differences at timepoints 18 ($p = 0.04$, $d_{rm} = 0.35$) and 22 ($p = 0.04$, $d_{rm} = 0.41$) compared to timepoint 0. No other significant differences were observed in sham including timepoint 14 ($p = 0.13$, $d_{rm} = 0.27$).

4.5.4 Discussion and Conclusion

We found that PACAP increased over the course of the stress protocol, an effect attenuated by tcVNS (but not sham stimulation) in traumatized individuals both with and without

PTSD. Increased PACAP concentrations were correlated with elevated PTSD symptoms at baseline, replicating earlier findings [101]. We also found that elevated PACAP was associated with increased symptoms of anxiety and depression and impairments in social and physical function. As PACAP is known to regulate cellular stress response [101, 102], longitudinal evaluation of PACAP may be helpful in tcVNS treatment monitoring.

This investigation touches on two main points. First, to our knowledge, this is the first report of PACAP in humans undergoing a trauma recall and mental stress paradigm over multiple days. Trauma recall and stressful tasks were associated with a steady increase in PACAP blood levels, regardless of the treatment status. Second, notably, the sham group's PACAP increase was higher, compared to active tcVNS group, which suggests that tcVNS may reduce PACAP elevation in response to stress. These results, along with significant correlations between PACAP and psychological scales (PTSD-SS, HAM-A, HAM-D, Baecke Sports Index, Baecke Leisure Index) suggest that PACAP may play an integral role in stress and PTSD, supporting relevant literature [102].

PTSD is also associated with poor health predictors, notably physical inactivity [331], also recognized in our study with negative correlations of baseline PACAP with Baecke Sports Index. We did not find significant correlations with other assessments of PTSD symptoms (CAPS, PCL-C, ETI, ATI), we believe that this could be due to small sample size. For example, only patients with PTSD can have a total CAPS score, which significantly decreased the sample size for this correlation. Regardless of the p-values, all psychological surveys indicating increased severity with higher scores were positively correlated with baseline PACAP. Similarly, physical activity scales (Baecke Questionnaire) were negatively correlated.

The limitations of this pilot study could be listed as follows. First, the sample included 12 patients with PTSD, and all three male patients were randomly assigned to the sham group. Due to this small sample size and the fact that none of the PTSD male subjects received the tcVNS treatment, we cannot evaluate the effect of tcVNS treatment on PACAP concentrations among PTSD males. Moreover, as gender may be an effect modifier of PACAP concentrations and PTSD, this interaction could not be evaluated within the tcVNS treatment group. Prior work has suggested that blood levels of PACAP are associated with PTSD diagnosis among females and stress-regulation pathways may vary between men and women [101]. Although levels of PACAP were lower at all times points taken after traumatic stress and mental stress in the tcVNS group compared to the sham group, the interaction term for device by time was not significant. This is also likely related to the small sample size, and therefore our results should be considered preliminary and in need of replication. PACAP is involved in circadian rhythm regulation [332-334]. Although our blood draws were scheduled in the A.M., our study lasted for three days, and the precise blood draw time for each subject varied somewhat, as would be anticipated with a multi-day human investigation. Lastly, in partial correlations between PACAP data (baseline, maximum, quartiles) and psychometric scales, we did not control for multiple testing, which may result in a Type I error. Again, this is considered an exploratory analysis, and results need to be established in a larger study.

In this study, we showed that acute traumatic and mental stressors are associated with increased PACAP concentrations in the peripheral blood in traumatized individuals both with and without PTSD. PACAP appears to be a modifiable biochemical biomarker, and its temporal changes may predict tcVNS treatment effect to acute stress or neuropsychiatric

disorders showing significant PACAP dysregulation. Moreover, longitudinal monitoring of PACAP may potentially be used to follow personalized, adaptive dosing strategies for larger trials and/or to identify respondent and non-respondent patients to potential treatments. Future studies should investigate sex differences in PACAP concentrations with acute and longitudinal tcVNS treatment using larger sample sizes.

4.6 tcVNS Reduces Stress-Induced Activation of Interleukin-6 and Interferon-γ in PTSD

We next examined the effects of tcVNS on peripheral cytokine response to the traumatic and mental stress protocol in traumatized subjects with and without PTSD. This part of the work was in collaboration with J. Douglas Bremner and Bradley D. Pearce's research group Emory University. Blood samples were processed at Bradley D. Pearce's lab by Allison Hankus. The measured cytokines were Interleukin (IL)-1ß, IL-2, IL-6, TNFα, and IFN-γ. These results are currently under review as a journal paper.

4.6.1 Statistical Analysis

ANOVA tests were used to compare the demographic characteristics across the tcVNS treatment or sham stimulation group among patients with PTSD and healthy participants. We used ANOVA and linear regression models to measure the association between the cytokine levels and PTSD status, with or without tcVNS treatment effect. The beta coefficients (ß) from the mixed models indicate the adjusted average percent or absolute differences in the changes of parameters from the corresponding rest values, comparing active vs. sham device types. ß were reported along with 95% CI and P-values. A two-

sided $p < 0.05$ denoted statistical significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and MATLAB (R2017b, Natick, MA).

4.6.2 Results

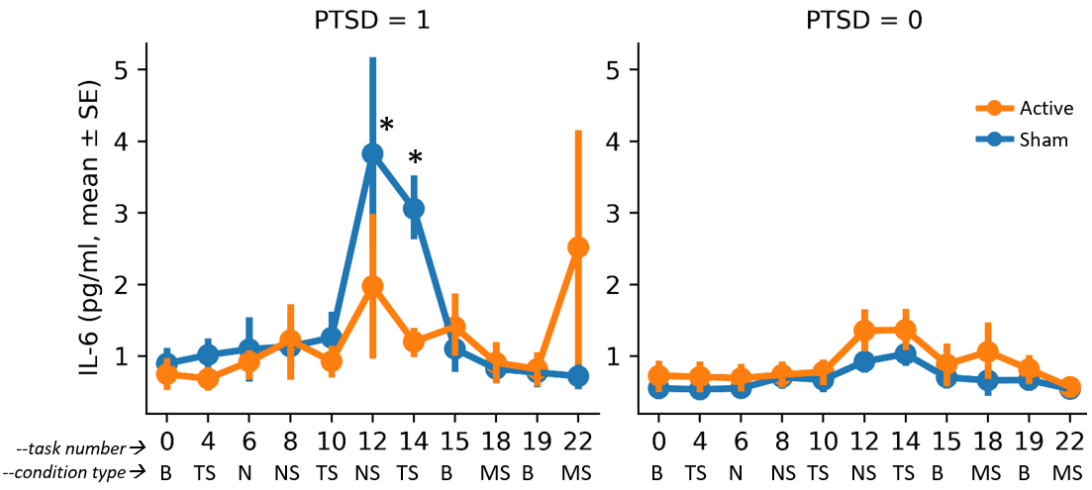

Figure 4-13. Effects of tcVNS or sham on IL-6 response to stress in patients with PTSD and traumatized participants without PTSD. Toward the end of day 1 with repeated traumatic stress (TS), there was an increase in IL-6 greater in sham versus tcVNS in PTSD patients (*) that occurred 90 minutes after the presentation of the first trauma scripts (Timepoints #12 and #14)($p < .05$). On day 2 participants underwent a baseline blood draw at rest (task #15) and 90 minutes after mental stress (MS) in the form of public speaking and mental arithmetic paired with tcVNS or sham (task #18). On day 3, participants again underwent a baseline blood draw at rest (task #19) and 90 minutes after mental stress (MS) using the same protocol as D2 (task #22). There were no significant differences between sham or active on days 2 or 3 with mental stress (MS, public speech and mental arithmetic) compared to each days' baseline in PTSD. Non-PTSD participants showed no difference between active or sham for either TS or MS days. Statistical analysis showed a significant day by diagnosis by device effect ($p < .05$), with secondary analysis showing a significant increase in IL-6 in sham versus tcVNS in the PTSD group with traumatic scripts (Day 1, $p < .05$).

The average age of this population was 30 (SD = 9), and the average BMI was 27 (SD = 5.60). Among all the participants, 18 (50%) were White / Caucasian, 21 (58%) were female, and consistent with prior reports [20], 9/12 (75%) of the PTSD patients were

female. All of the PTSD participants randomized to VNS were female (Fisher's Exact p = 0.045), and the gender proportion in the other groups was similar.

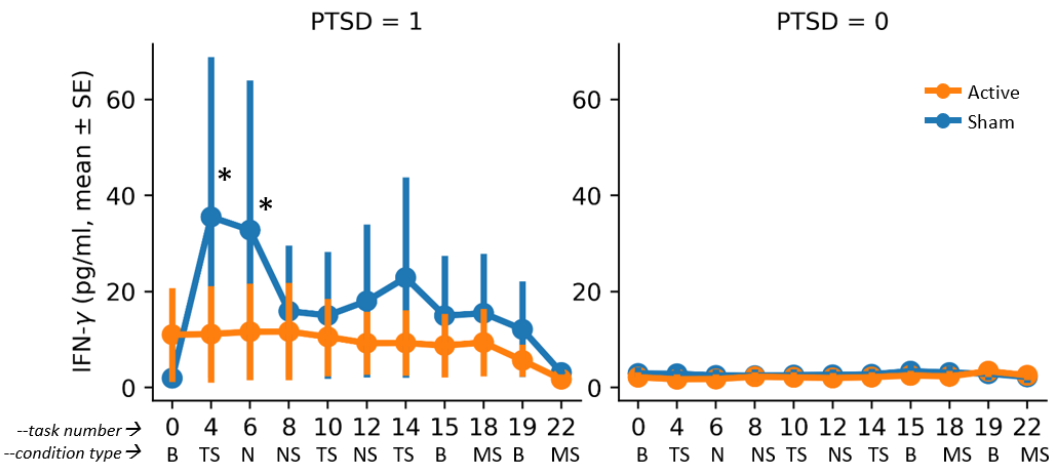

Figure 4-14. Effects of tcVNS or sham on IFN-γ response to stress in patients with PTSD and traumatized participants without PTSD. Overall there was a marked increase in IFN-γ in the PTSD but not the non-PTSD participants which was most pronounced after the first traumatic script (task #4) and was largely blocked by tcVNS but not sham, resulting in a significant increase in IFN-γ over the three day stress protocol in the sham group versus active tcVNS (*, $p < .05$).

Exposure to personalized traumatic scripts in conjunction with sham stimulation resulted in an increase in IL-6 in PTSD but not non-PTSD participants that was greater following repeated exposure to personalized traumatic scripts (Day 1) than for mental stress (public speaking and mental arithmetic on Days 2 and 3), that peaked about 90 minutes after exposure to the first traumatic scripts and was blocked by tcVNS (ß = 0.474, 0.009 – 0.939 95% CI, p = 0.046) (Figure 4.13). There was minimal effect on IL-6 for mental stress (public speaking and mental arithmetic) on days 2 and 3 in either the PTSD or non-PTSD, sham or tcVNS groups. Personalized traumatic scripts resulted in an immediate and marked rise in IFN-γ on Day 1 in the PTSD but not the non-PTSD participants (Figure 4.14). The traumatic script-induced increase in IFN-γ was blocked by tcVNS versus sham (ß = -0.246,

-0.470 -- -0.022 95% CI, p = 0.032) (Figure 4.14). There were no statistically significant differences between tcVNS of sham stimulation groups in IL-2, IL-1ß or TNF-α.

4.6.3 Discussion and Conclusion

tcVNS blocked an increase in the inflammatory marker IL-6 and IFN-γ seen with personalized traumatic scripts in PTSD patients administered sham stimulation. Non-PTSD participants with a history of exposure to psychological trauma overall had minimal IL-6 or IFN-γ increases in response to personalized traumatic scripts. Personalized traumatic scripts had much greater effects than mental stress including mental arithmetic and public speaking on IL-6 and INF-γ in PTSD patients and therefore the blocking effects of tcVNS were more prominent.

Studies showing that peripheral IL-6 and TNF-α concentrations vary with changes in HRV are consistent with the current findings that the vagus modulates peripheral inflammation [335, 336]. The current study shows that PTSD patients have an enhanced inflammatory response to stress, with the greatest effects for personalized traumatic scripts. Our finding of blocked IL-6 and IFN-γ responses to stress with tcVNS adds to the growing literature on nVNS affecting central brain and peripheral autonomic function in human [160, 161, 174, 178, 293, 329, 337, 338] and animal studies [177, 181, 182].

Projections of the vagus through the nucleus tractus solitarius (NTS) extend to the locus coeruleus and hypothalamus, key areas involved in sympathetic hyperarousal in PTSD, as well as brain areas like the amygdala that are involved in the fear response and the medial prefrontal cortex / anterior cingulate, which is involved in both fear extinction and modulation of peripheral neurohormonal responses to stress . tcVNS likely travels through

these central pathways to effect changes in peripheral inflammation. Cytokines, inflammasomes, and other inflammatory markers have behavioral effects similar to stress-related psychiatric symptoms [69, 339], so reduction of spikes in IL-6 and IFN-γ that likely occur multiple times a day with traumatic reminders and daily stressors in PTSD patients to will likely benefit symptoms driven by inflammation and lead to improvements in clinical course. For instance, in our studies of coronary artery disease [62] patients, we found not only an increase in mental stress-induced IL-6 in those with co-morbid PTSD [99]but also that psychological distress (including an aggregate measure of subjective anger, distress and PTSD) was associated with long-term adverse cardiovascular outcomes [340]. Furthermore, CAD patients with mental stress-induced myocardial ischemia (MSI) had an increase in PTSD [340], and subjective anger response to stress was associated with MSI [64].

IL-6 and IFN-γ represent only one part of a complex system that is responsive to stress and could be modifiable by tcVNS. Other inflammatory factors implicated in PTSD include High mobility group protein B 1 (HMGPB1), a proinflammatory master mediator, which is increased in PTSD, and inhibited by VNS [341, 342]. T helper (TH) cell differentiation is partly controlled by cholinergic neurotransmission and therefore amenable to vagal stimulation [343], and dysregulation of TH cells differentiation and function has been proposed in PTSD [344]. TH1 cytokines include proinflammatory mediators (IFN-γ) as well as IL-2 and IL-3. TH2 cytokines are IL-4, IL-5, and IL-13. IL10 is stimulated by catecholamines, is broadly anti-inflammatory, and induces a shift in the TH1/TH2 balance toward TH2 dominance. Although both afferent and efferent arms of the vagus may contribute to the reduction in these proinflammatory cytokines, selective unidirectional

stimulation of the cervical vagus has been shown to dampen TNF production [345]. The kynurenine pathway is also relevant to the effects of VNS on physiological function and mental disorders. VNS in humans resulted in a tendency to a reduction in kynurenine [137], and an increase in anthranilic acid (AA), which is neuroprotective . Kynurenic acid (KYNA) is an antagonist of alpha-7 Nicotinic receptors, which are key mediators of the efferent cholinergic anti-inflammatory loop [343, 345, 346].

This investigation is not without limitations. The sample size was small, and the current findings should be replicated in other samples. The current study also did not find as much of an IL-6 response to mental stress as in our prior study of public speaking stress in patients with PTSD. That was a different sample, however, including older patients with CAD and more medical comorbidities than the current sample of younger uncomplicated PTSD patients [99]. The prior study was also the first exposure to stress performed while sitting in a chair as opposed to lying in a scanner, which we have found presents a more direct interpersonal experience in the solicitation of stressful responses within a social context. In prior studies we found a reduction in cardiovascular reactivity on a following day when stress was repeated in a scanner [347]. In the current study, neutral mental stress came on subsequent days to personalized traumatic scripts, so a reduction in responsiveness is to be expected.

4.7 Self-Reported Anger Response for tcVNS Paired with Traumatic Recall

We next examined subjective anger scores, grouped by PTSD and non-PTSD. The questionnaires were performed by the clinical staff during the three-day protocol. Non-PTSD participants had minimal anger responses with no statistically significant differences

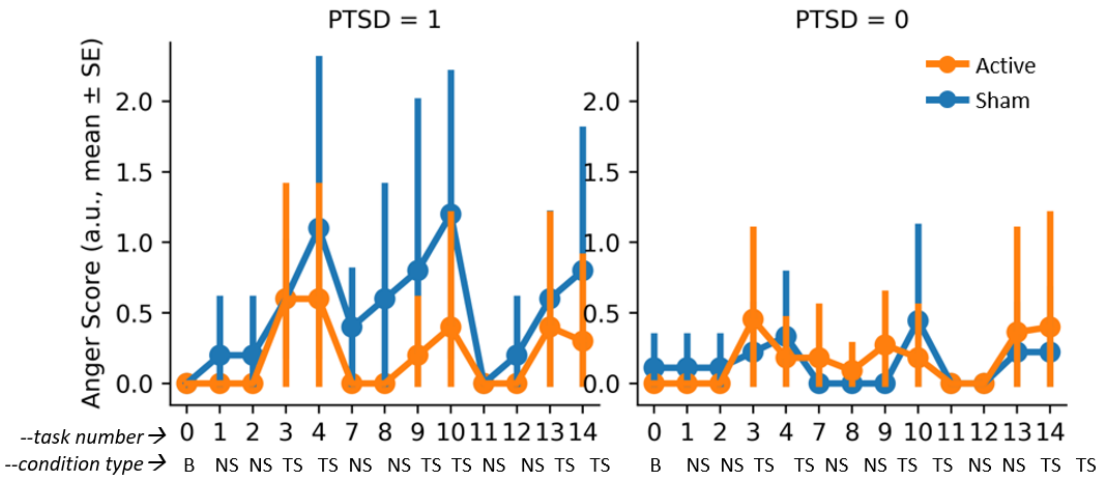

Figure 4-15. Effects of tcVNS or sham on subjective anger as measured with the Visual Analogue Scale (VISAN). There were increases in anger over time from baseline (B) and neutral scripts (NS) to trauma scripts (TS) in PTSD that were blunted by pairing with active tcVNS. Traumatized participants without PTSD showed less of an anger response to scripts and did not show differences between groups.

between the tcVNS and sham stimulation groups (Figure 4.15). It is notable that PTSD patients who received active tcVNS reported less subjective anger scores, although active and sham groups started with similar anger rating at the very first traumatic script (no stimulations before). These data highlight potential beneficial effects of tcVNS in perceived anger, however a larger sample is necessary to draw behavioral conclusions.

4.8 Automatic Detection of Target Engagement for tcVNS

Transferring tcVNS technologies to at-home settings brings challenges associated with the assessment of therapy response. In this part of the investigation, we studied how machine learning can help to determine target engagement for tcVNS using noninvasively obtained ANS activity information [329]. We created a feature set from autonomic parameters to: 1) detect active (vs. sham) tcVNS stimulation presence with machine learning methods,

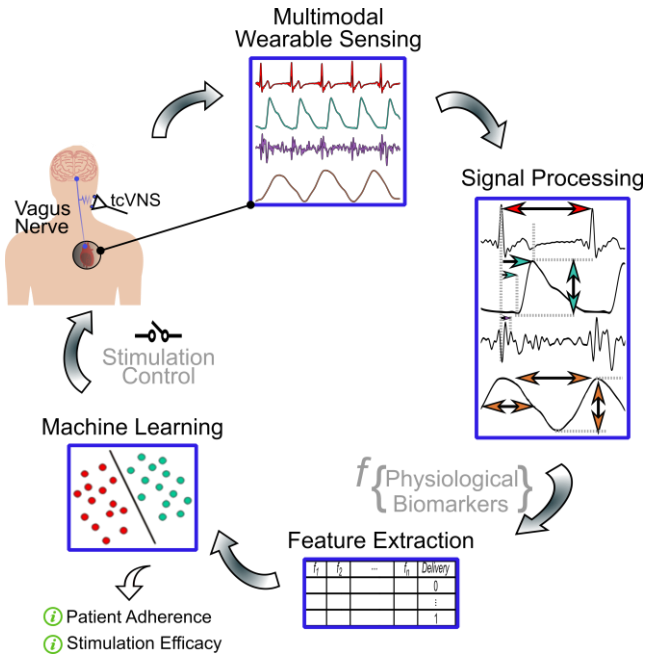

Figure 4-16. Wearable neuromodulation technologies can interface with noninvasive sensing. Ongoing activity acquired from one or more sensors can be utilized to assess cardiovascular and peripheral physiological response via signal processing. Events or changes in these data streams can then be decoded using machine learning via feature extraction, in order to dynamically trigger closed-loop delivery when needed, and to quantify patient adherence and stimulation efficacy for at-home therapy.

and 2) determine which sensing modalities and features provide the most salient markers of tcVNS-based changes in physiological signals.

The ANS activity data with tcVNS applied in tandem with acute traumatic stress is clinically relevant. Patients with trauma-related psychiatric disorders may experience traumatic flashbacks multiple times a day, triggered by, for example, sensory data (odor, sound, room size), cues related to the traumatic event, or even temporal data (incident repeated at the same time of the day) [348-352]. These specific triggers may be relevant to personal traumatic memories from sexual assault to combat exposure [13]. Engineering efforts to transfer tcVNS to at-home use bring challenges regarding the stimulation

application. A fundamental challenge is the determination of whether stimulation delivered properly [353]: The electrode placement and stimulation intensity affect the current flow and distribution, hence the stimulation efficacy, as tcVNS electrodes are directly in contact with the skin rather than placed on the nerve itself (as with an implantable counterpart). Thus, personalized neuromodulation to provide feedback for the physician or for the patient on whether stimulation has been properly delivered to the vagus nerve is necessary for longitudinal treatment paradigms. As summarized in Figure 4.16 interfacing tcVNS with noninvasive sensing modalities and extracting information to decode whether the stimulation engaged the nerve target could dramatically improve stimulation efficacy and provide closed-loop delivery in response to a detected event. Continuous cardiovascular and peripheral physiological sensing offer a convenient tool for this investigation, due to the intimate relationship of the vagus with the heart and the peripheral physiology, and the ubiquity of noninvasive wearable technologies. Previously, we observed changes in individual features such as HR, the differences between groups in these individual features were not sufficient to allow for classifying whether or not tcVNS was properly delivered. Another point to consider is the optimization of the acquired modalities: while wearable sensing offers a convenient tool to quantify tcVNS, learning from the optimized modalities (i.e. "learning from less data") would be favorable for hardware adaptations that possibly may not employ all measurement modalities discussed.

In this work, we employed machine learning techniques to determine the stimulation type, active or sham, from the extracted ANS activity parameters and determined suitable sensing modalities for "in the wild" tcVNS use that could quantify therapy response, and ultimately enable closed-loop tcVNS delivery when a traumatic stress trigger is detected.

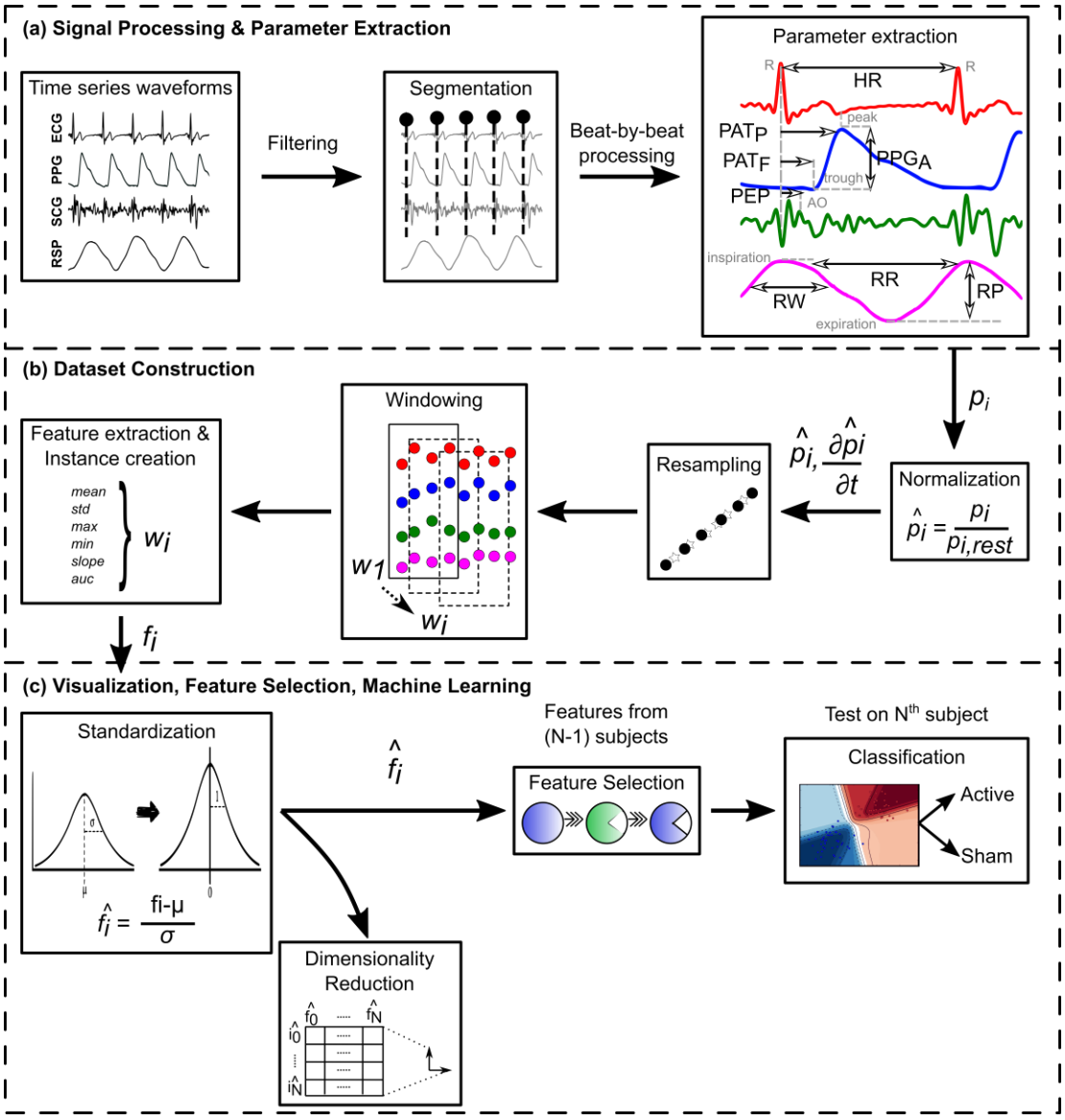

Figure 4-17. a) ECG, PPG, SCG, RSP signals were processed and HR, PAT, PEP, PPG amplitude, RR, RW, RP were extracted as physiological parameters. b) Using the extracted parameters, dataset constructed after normalization, resampling, and windowing. c) After standardization, dimensionality reduction methods were applied for dataset visualization. Then, feature selection and machine learning were conducted. PAT_F: PAT_{FOOT}; PAT_P: PAT_{PEAK}; AO: Aortic opening; PPG_A: PPG amplitude.

4.8.1 Statistical Analysis

Subject demographics (age, gender, height, weight, body-mass index) and baseline physiological parameters were compared between the two device groups, active and sham, to understand whether the groups were significantly different from each other. Normality was assessed using Shapiro-Wilk test. The comparisons were made using t-tests for normal continuous variables, Wilcoxon rank-sum tests for non-normal continuous variables, and chi-squared tests for categorical variables. P-values lower than 0.05 were considered statistically significant. The groups were determined to be balanced with equivalent baseline characteristics, rendering the data amenable to use for further engineering purposes, demographics detailed in Ref. [329].

4.8.2 Dataset Construction

Figure 4.17 summarizes signal processing and parameter extraction paradigm, conducted in MATLAB (R2017b, Natick, MA). The physiological parameters related to ANS and peripheral physiological activity were extracted from four sensing modalities: ECG, PPG, SCG, RSP. The physiological parameters were extracted from stimulation and post-stimulation intervals, which correspond to the data from the last minute of stimulation and one-minute data from three minutes after the stimulation ended, respectively. Then, these parameters were divided by the mean values obtained from the rest period to normalize inter-subject variability. The rest data were obtained before the stimulation protocol started, at the same body position the stimulation was carried out. The dataset for machine learning was constructed from the time-series data obtained from the normalized intervals. The extracted parameters were resampled to the length of the parameter that has the maximum length to equalize the length of each parameter within an interval for further usage of the resampled data in windowing (to obtain equal length of arrays for windowing),

using an antialiasing FIR lowpass filter and compensating for the delay introduced by the filter. Resampling was necessary as the length of respiratory features are approximately half of the beat-by-beat features per unit time, as human respiration and heartbeat frequency are different (12-20 breaths per minute respiratory rate versus 70-100 beats per minute heart rate). Then, instances were created by using 10-sample sliding windows with 9-sample overlap (90% overlap). The features consisted of the mean, standard deviation (std), maximum (max), minimum (min), area under curve (auc) and slope (slp) of the extracted physiological parameters in each window. The approximate first order derivatives (differences between the adjacent elements) were also computed to generate additional parameters. The same feature calculation methodology was applied to the difference matrices (except slope). The multi-dimensional feature matrix was constructed from all extracted features and the corresponding labels as device types (tcVNS, sham). This matrix included features as columns and instances as rows, consisting of 176 features and 1780 instances (827 tcVNS, 953 sham). Overall, these features were derived from 16 time-series data (HR, PEP, PPG Amplitude, PAT_{FOOT}, PAT_{PEAK}, RR, RW, RP in two intervals), with 11 mathematical properties derived from each of them and their differences. Later, the data were standardized across each feature to have a mean of zero and a standard deviation of one.

4.8.3 Machine Learning

For the machine learning paradigm, our primary concerns were 1) minimizing the number of features/sensing modalities used in classification to optimize the computational power and to determine which sensing modalities are necessary for a wearable implementation (note that there are a total of 176 features from four sensors with unknown contributions to

the classification task), 2) developing a realistic validation paradigm for a new subject who has just started undergoing stimulation. To address 1, we implemented a univariate feature selection paradigm based on sorting ANOVA F-statistics of features to eliminate redundant data without incurring significant loss of information [221]. Note that this feature selection step is not mandatory but optional. Assuming no need for optimization, not applying this step (simply using all features) did not cause a dramatic decrease in performance (See Table 4.2, Support Vector Machine with sigmoid kernel, which was used as our classifier). To address 2, we wanted to ensure that the training dataset are not biased for a new, incoming subject by using LOSO-CV. Typical methods such as K-fold cross validation would not guarantee that the training dataset does not have data from the incoming subject. The fact that there is data from the incoming subject in both the training and testing datasets will likely make the model know more about the target subject than it should. With an entirely new subject (not in the training dataset), the K-fold trained model will potentially perform poorly because it did not include data from the new subject in the training set before. LOSO-CV, on the other hand, guarantees there is no data from the incoming subject in the training dataset in model evaluation process already, hence this is a conservative approach to assess an expected performance for an incoming subject. Feature selection and LOSO-CV were implemented as follows: For each LOSO-CV loop, one subject was left out of the feature selection and classification model training, then used for testing. The procedure was repeated for each of the 26 subjects.

Table 4-2. ROC AUC Scores of Classifiers Derived Using LOSO-CV and All Features

Naïve Bayes	**Random Forest**	**k-NN**	**Logistic Regression**	**MLP**
0.52	0.76	0.56	0.80	0.71
Decision Trees	**SVM (Linear)**	**SVM (Polynomial)**	**SVM (RBF)**	**SVM (Sigmoid)**
0.62	0.80	0.66	0.65	0.98

ROC AUC = Receiver operator characteristics area under curve; k-NN = k-Nearest Neighbors; MLP = Multilayer Perceptron; SVM (Kernel) = Support Vector Machine (Kernel Function); RBF = Radial Basis Function

We determined the number of top features by implementing the feature selection and LOSO-CV and swiping the number of top features from 1 to 176. For each case, we computed the area under curve obtained from the receiver operator characteristics (ROC AUC) and plotted $k_{topfeatures}$ versus ROC AUC. Then, we determined a range for $k_{topfeatures}$ where ROC AUC is high (> 0.9) and robust enough to ensure good performance and $k_{topfeatures}$ is low enough to reduce processing time.

Following the determination of feature selection and the needs for model evaluation, we explored the most effective classification architecture for predicting stimulation presence from physiological signals. Using LOSO-CV, we used the following classifiers: Naïve Bayes (for predicting baseline performance), Random Forest, k-Nearest Neighbors (k-NN), Logistic Regression, Multilayer Perceptron (MLP), Decision Trees, and Support Vector Machine (SVM) with linear, polynomial, radial bases function (RBF), and sigmoid kernels.

Table 4.2 lists the ROC AUC values for each classifier. We observe that SVM with a sigmoid kernel performs the best among different classifiers used, hence we decided to use this classifier.

4.8.4 Results

We performed multiple classification runs with a sigmoid SVM classifier using features from different sensors alone and their combinations. Supplementary Figure S1 in [329] shows the number of features versus ROC AUC, plotted from one feature to the maximum number of features based on the sensors chosen. It is apparent that ROC AUC>0.8 could be obtained from different sensor combinations.

By using the described feature selection and machine learning methodologies, we obtained the highest ROC AUC (> 0.90) and highest accuracy, precision, recall (> 0.90) for four cases: By using: 1) all sensors, at least 140 top features out of 176; 2) ECG and PPG sensors, at least 70 top features out of 88; 3) ECG, SCG, and PPG sensors, at least 99 top features out of 110, and 4) ECG, PPG, and RSP sensors, at least 84 features out of 154. As our aim was to minimize the number of sensing modalities and features to reduce complexity for a future wearable implementation, we focused on the classification results for the second case, the combination of ECG and PPG. The features in this dataset contain HR, PPG amplitude, and PAT.

Our next goal was to analyze the results obtained from ECG and PPG. Figure 4.18 details the outcomes for the features obtained from ECG and PPG. Figure 4.18a shows the t-SNE plot grouped by tcVNS and sham clusters. A nonlinear separation exists between the device groups. Figure 4.18b shows how the number of selected top features change the ROC area.

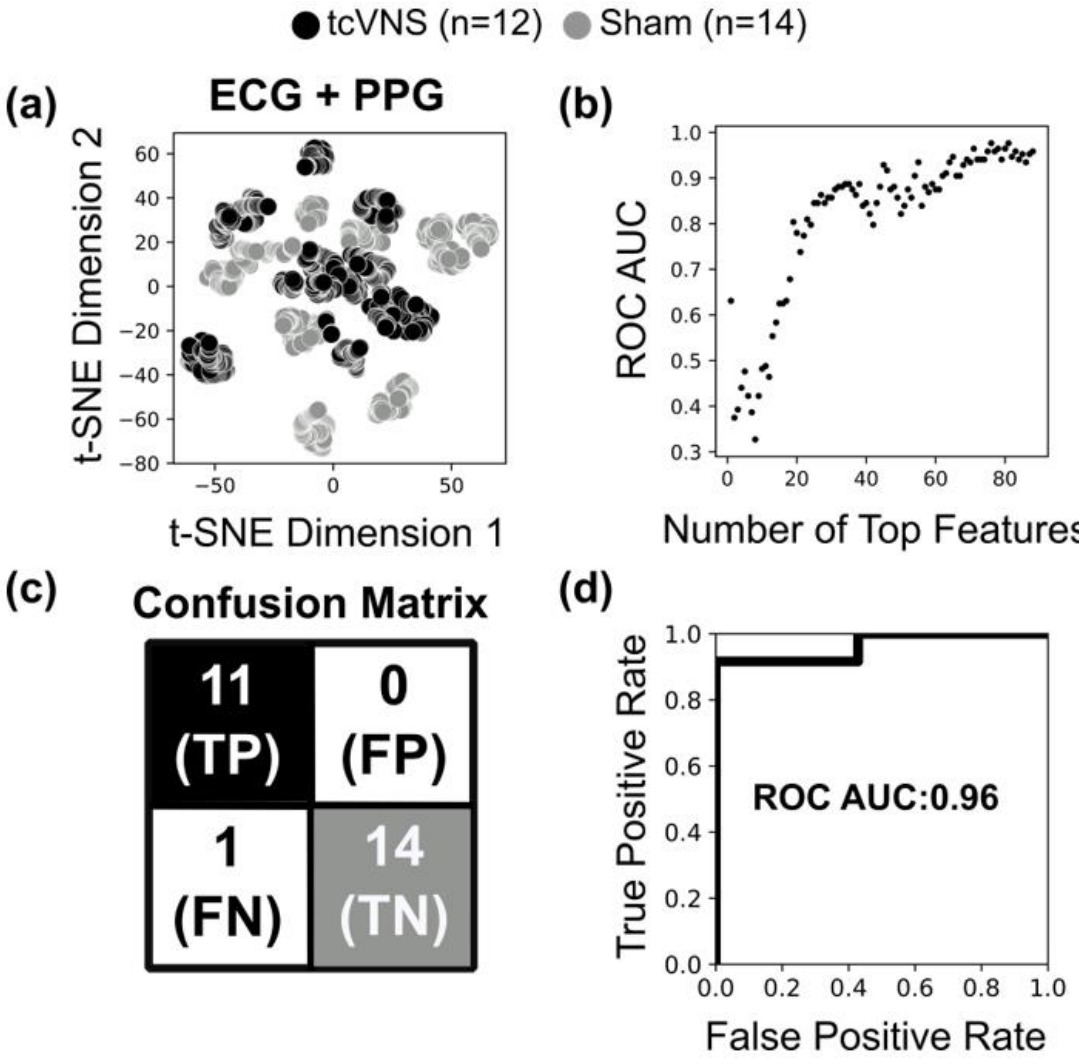

Figure 4-18. Dimensionality reduction and classification outcomes for separating the stimulus types: active tcVNS and sham. a) Dimensionality reduction applied to the high-dimensional feature matrix using t-SNE constructed from features from ECG and PPG. b) Number of Top features selected using ANOVA F-score-based feature selection versus receiver operator characteristics (ROC) area under curve (AUC). ROC AUC is robust to Top Features from 70 to 88. c) Confusion matrix for the classifier, obtained with LOSO-CV and minimum number of features (71). d) Receiver operator characteristics (ROC) for the classifier. A ROC area under curve (AUC) of 0.96 was obtained. Classification outcomes vary minorly with Top Features from 70 to 88.

A ROC area of > 0.9 can be obtained by using 70 top features out of 88 (and beyond).

Figure 4.18c-d summarize the machine learning outcomes for the classification using top

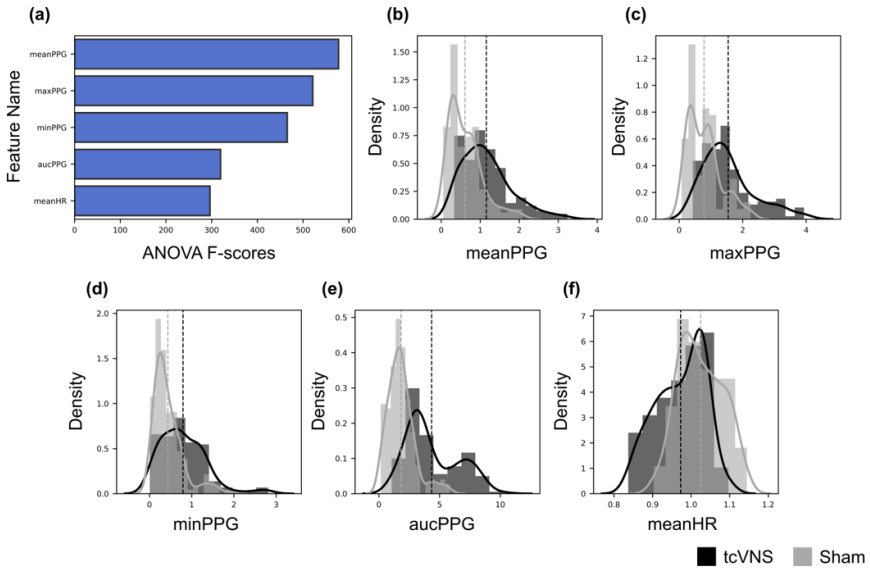

Figure 4-19. Top five features sorted by ANOVA F-values, their histograms, and kernel density estimates grouped by classes. The top features were calculated from the full feature set obtained from ECG and PPG sensors. The dashed lines indicate the mean of the class.

71 features out of 88 (note that outcomes are similar between 70 to 88). The classification resulted in 25 correctly classified subjects out of 26 total subjects (12 active tcVNS, 14 sham) with LOSO-CV. There was one false negative subject. Macro-averaged accuracy, precision, recall, and F1 scores of 96% with 0.96 ROC AUC were obtained as the performance outcomes. Due to the feature selection method applied in each LOSO-CV loop, the features used in the classification slightly differed for each subject. Figure 4.19 shows the top 5 features obtained by applying the feature selection method to the whole dataset of 88, sorted by ANOVA F-values. PPG and HR features resulted in the largest F-values. Lastly, we calculated the time complexity (the time elapsed encompassing all tasks ranging from signal processing to machine learning) for each subject as measured on a Core i7-6500U CPU @2.5GHz 12GB RAM personal laptop. We found that 2.6 (1.1) seconds (mean (SD)) were required to generate an output class for an incoming subject.

4.8.5 Discussion and Conclusion

In this work, we investigated methods for detecting target engagement for tcVNS by using cardiovascular and peripheral signals. The dataset constructed from ECG and PPG signals yielded sufficient information to detect whether therapy response occurred (active device), resulting in 25 correctly classified subjects out of 26. The false negative subject (active subject classified as sham) was a female aged 24 years. The combination of other sensing modalities resulted in similar classification outcomes, slightly changing the confusion matrix. Single sensors/features do not offer the same performance as the combinations. Thus, there is not a single biomarker or pattern that could be easily recognized by the human eyes; there is likely a complex, high-dimensional relation between multiple features that requires multiple modalities.

The investigation of the most salient features reveals that features related to PPG and HR perform favorably compared to others. HR is regulated by both SNS and PNS activity [38]. Stimulation of the vagus nerve typically decreases SNS activity or increases PNS activity, also observed in the clinical portion of this study [293]. Moreover, increase in PPG amplitude was noted when tcVNS was paired with traumatic stress, compared to sham. From Figure 4.19b-f, PPG features increased and mean HR decreased for the tcVNS group. Both of these changes indicate a decrease in sympathetic tone, which perhaps makes the separation between the classes possible by these features.

The analysis of machine learning performance outcomes is essential to gain an understanding regarding the translation of the methods to at-home settings: with our classifier, the accuracy, precision, recall, F1 scores were 0.96. Thus, the classifier could

provide a binary (e.g., red or green) indicator to the user following stimulation regarding whether the nerve target was successfully engaged. Among 100 stimulation administrations upon traumatic stress triggers, we would be able detect whether the stimulation happened correctly 96 times.

The methods described in this study leverage physiological signals that are convenient to obtain with wearable sensing modalities, such as the smartwatches that measure ECG and PPG. The framework presented herein could remain the same and could be generalized to include other types of sensory data. Note that although the measured computation time was 2.6 seconds on average to generate an output class for an incoming subject, this was computed with a personal laptop. A final implementation of this pipeline would not require a fully embedded platform as no precision timing is necessary. The collected signals could be transferred to a Cloud-based distributed system that employs parallel processing capabilities to further reduce this 2.6-second computation time if needed.

Among the modalities we used, HR and respiratory effort information are typically collected in clinical practice, in a non-continuous manner. Therefore, we explored using only these features. Using the features obtained from HR, RSP, and their combination for the classification task do not outperform the reported results. The reader is referred to Supplementary Figure S1 in [329] for the details of this result.

Other measures that might indicate stimulation presence from the literature are the use of microneurography, pupil size, serum cytokines (anti-inflammatory effects as quantified by tumor necrosis alpha, interleukin-6), HRV, or evoked potentials resulting from stimulation [157, 178, 354, 355]. Microneurography and serum cytokines are not non-invasive, not

pursuant to the goal of real-time identification, and are confined to clinical settings, electroencephalogram requires bulky multi-channel equipment, while pupil size was not found to give favorable results for stimulation in prior work. As for HRV, this measure does not have a continuous nature and requires a long-term clean recording to compute a single number based on the variability in R-R intervals. An auricular stimulation study noted changes in HRV with long-term recordings [157], while some studies did not note changes in HRV [356].

A number of limitations exist for the study described here. First, this study employs a reactive approach. Traumatic stress requires a flashback occurrence: the subjects should remember their memories to be stressed, which requires some time starting from the introduction of the traumatic recording. We applied stimulation immediately after the traumatic stress recording as our prior brain imaging studies demonstrated that the arousal persists following rumination of the traumatic script recording [51, 304]. Future studies should consider sweeping the timing of the stimulation, which might possibly downregulate the autonomic reactivity even before reaching a peak stressed state, based on prior preclinical studies [145, 303]. Second, this study did not have continuous blood pressure recordings, hence we could not use blood pressure-related measures except pulse arrival time (PAT) obtained from ECG and PPG, which is a measure related to both continuous blood pressure and cardiac contractility [206]. Nevertheless, analyses on the effects of auricular or cervical stimulation on cardiovascular and autonomic function have produced mixed outcomes throughout many studies that use basic vital signals such as HR, HRV, or BP, hence these basic measures are not likely to be sufficient for monitoring stimulation presence [296, 297, 356]. Lastly, in the current study, we obtained a

transmissive PPG signal that requires the photodiode (PD) and light emitting diode (LED) combination to be at the opposite sides of the skin. A reflective PPG sensor (both PD and LED on the same side of the skin) might be more appropriate for a comfortable and minimally obtrusive wearable device.

This study demonstrates the first effort to provide real-time inputs that could be used in tcVNS therapy response, and further for closed-loop modulation for at-home tcVNS technologies. Multimodal signal fusion might be a viable approach in determining whether the stimulation occurred as expected. In addition to the investigation of individual parameters, sensor fusion could be instrumental in translating tcVNS to unsupervised settings to improve therapy response in a home-based setting. ECG and PPG sensing appear to provide relevant information regarding the stimulation delivery, and both signals could be obtained noninvasively, and are also prevalent in commercially available wearable sensing devices. The methods presented herein could thus be deployed in wearables allowing for a convenient home-based approach to supporting accurate and effective delivery of noninvasive vagal nerve stimulation therapies.

Future studies should focus on the following to facilitate successful translation to clinical practice. Continuous blood pressure should be measured as an additional physiological parameter to be studied as it includes important vascular information; effects of the time of stimulation (before or during traumatic stimuli) on efficacy should be determined; and the ability of indices derived from reflective PPG sensors to capture the key physiological information should be quantified. Additionally, stimulation induces parameter-specific changes in physiology [155]. Therefore, regression models of stimulation parameters onto

the changes in cardiovascular and peripheral measures could provide a better understanding for inter-patient variability in noninvasive VNS studies.

Finally, as another dimension of analysis, the positron emission tomography (PET) imaging part of the study led by Matthew T. Wittbrodt (Emory School of Medicine) was also recently published [357]. The analysis investigated the brain activity during traumatic stress for the non-PTSD sample. The results suggest that, compared to sham group, active tcVNS decreased brain activity during traumatic stress by attenuating the increased neural activity in the fear memory circuitry. In addition, patterns of decreased activation for the active group suggest improved appraisal and emotional processing.

In summary, this chapter presented a broad investigation involving engineering, basic science, and clinical research on the use of tcVNS to mitigate the body's acute stress response in a double-blind sham controlled randomized study. tcVNS appears to modulate physiological, inflammatory, and cellular responses to emotional and mental stress, as well as self-reported mood scales signal an attenuation in perceived anger in individuals with or without PTSD. Physiological measures quantifying the autonomic state are powerful candidates for technological translation, as they do not require blood work, could be obtained in remote settings outside of clinics, and are amenable to be designed as closed-loop systems satisfying rehabilitation upon the detection of an autonomic perturbation.

CHAPTER 5. OBJECTIVE PTSD ASSESSMENT THROUGH TRAUMATIC STRESS REACTIVITY

In this chapter, a methodology based on autonomic reactivity to traumatic reminders for objective assessment of PTSD is introduced. This methodology relies only on objectively measurable psychophysiological activity and patient background, rather than structured interviews prone to malingering and under-reporting. We consider a holistic approach, fusing autonomic reactivity to traumatic reminders—captured with multiple modalities of physiological sensors—and patient background. This assessment could help objective diagnosis of PTSD and potentially long-term monitoring of PTSD therapy response or trauma recall reactivity. Additionally, this work substantially extends the current literature by introducing psychophysiological correlates of clinician administered psychometric, personality, mood, and discomfort scales.

5.1 Introduction

PTSD may develop following single or repeated exposure to trauma related to threat of death, violence, or injury, either by direct exposure or witnessing/learning someone close to the individual faced the event. The current state of the art for diagnosing PTSD requires interaction with a medical professional and an approximately one hour long structured interview. As the diagnosis has a self-report nature, the interviews are susceptible to malingering or under-reporting the specific categories [183, 184]. Moreover, there is now a plethora of evidence on the effects of traumatic stress on survivors: Patients with PTSD experience a broad range of problem with memory, including problems with remembering

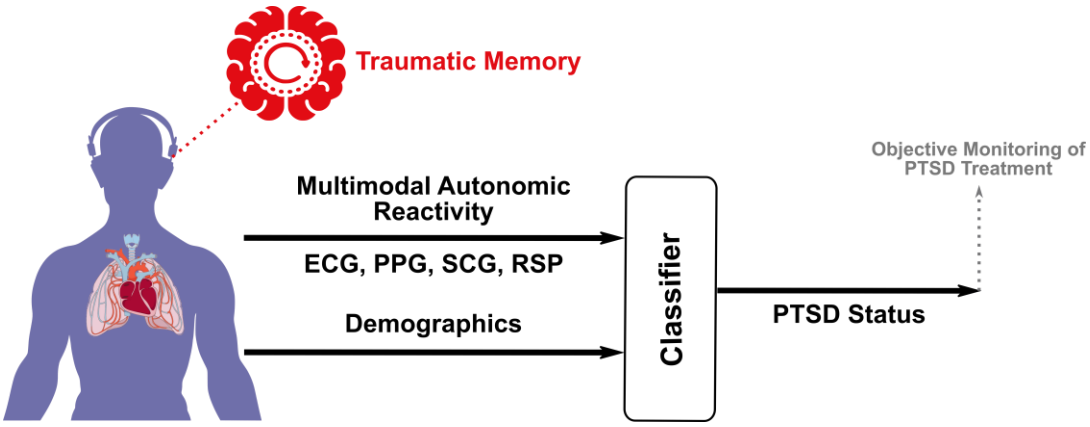

Figure 5-1. Illustration of reactivity-based objective PTSD assessment scheme. The fusion of autonomic reactivity to traumatic reminders and patient demographics could help objective diagnosis of PTSD and long-term monitoring of PTSD therapy response. Autonomic reactivity information is obtained using noninvasively obtained physiological signals germane to wearable sensing devices while the patient is hearing personal traumatic memories.

the traumatic event, gaps in memory, or in contrast, intrusive memories, evidenced by both preclinical and clinical research [358-361]. Given these, more objective measures are needed to assist in PTSD diagnosis.

Previous research suggests that baseline physiology such as resting skin conductance and resting heart rate are informative: patients with PTSD exhibit greater levels in magnitude than those without PTSD [362, 363]. In addition to baseline physiology, patients with PTSD are known to elicit elevated psychophysiological reactivity to trauma-related cues [364]. Increased reactivity in HR, EDA, facial electromyogram (EMG), and HRV are observed as psychophysiological measures [365, 366]. Reactivity to trauma-related cues are associated with the severity of disorder, either for standardized trauma-related stimuli or internal mental imagery of traumatic events [364]. A recent study used trauma-related virtual reality videos to assess PTSD status using heart rate and skin conductance

responses, obtaining classification accuracy between 89% to 92% (PTSD/non-PTSD) using 58 male participants with military service experience [367]. While promising, lack of women in the datasets limited the generalizability of the results. Another study used a novel method, speech samples, from male, warzone-exposed veterans with and without PTSD during CAPS interview [368]. Similarly, the dataset included only males who were already undergoing a CAPS interview, hence the usability of the technique and applicability to women have been open questions, though with promising results. Women are twice more likely to experience PTSD than men: 10% of women have PTSD sometime in their lives compared to 4% in men [369, 370]. In addition, more civilians are likely to experience PTSD than veteran population [371, 372], though the exposure likelihood increases in veterans.

In this work, we utilized our dataset from physically healthy human individuals (females and males) with prior exposure to psychological trauma but without PTSD (n = 26), and patients with PTSD (n = 25) while they underwent a one-minute trauma recall paradigm to identify PTSD status. We developed a holistic approach using multiple measures of autonomic nervous system activity and patient background as a potential objective measure of PTSD (Figure 5.1). This is the first study to identify autonomic activity and patient background features that differentiate PTSD from trauma-exposed individuals without PTSD. We also correlated psychophysiological reactivity to traumatic memories with current standard psychometric scales.

5.2 Methods

Figure 5.2 shows the CONSORT and dataset diagram detailing the available data for 51 human subjects. Available and categorized data categories were: demographics, baseline psychometric scales, baseline personality scales, visual analogue scales (baseline and trauma), blood pressure (baseline and trauma), physiological parameters, and trauma details. Tables 5.1 and 5.2 show the demographics (age, sex, height, weight, BMI, education, race), psychometric scale scores (PCL-C, CAPS, PTSD-SS, HAM-A, HAM-D, CADSS, BDI, ETI, ATI, ESSI), baseline personality surveys (Big Five, Toronto Alexithymia Scale), baseline mood scales (distress, VISAN) broken into PTSD and non-PTSD groups. As trauma research literature suggests that the trauma details might be important in psychophysiological reactivity, we also categorized each subject's traumatic experience. Trauma type (child abuse/neglect, sexual/physical assault, combat, other), existence of sexual content (yes/no), trauma time (childhood, adulthood), trauma onset (> 6 months, < 6 months) were categorized based on clinical assistance and relevant literature [364, 373, 374]. We performed a number of analyses: 1) For understanding the basic differences between groups, we first compared the aforementioned information between PTSD and non-PTSD groups. 2) For understanding the associations between physiological variables and psychometric scales/PTSD status, we carried out partial correlations using

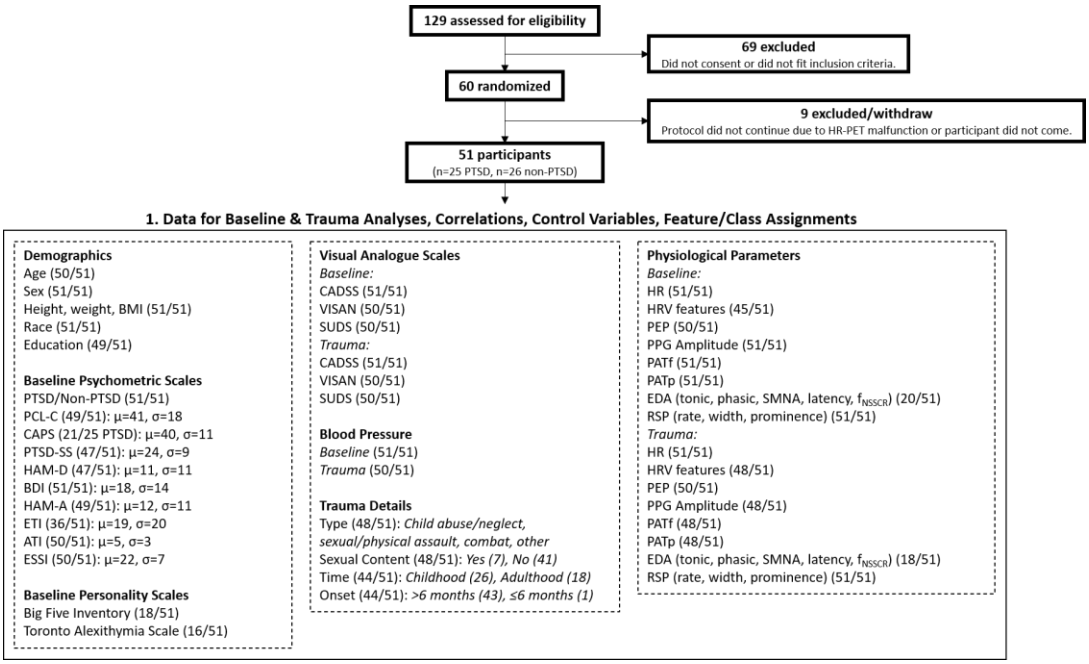

Figure 5-2. Available demographics, psychometric scale, personality scale, visual analogue scale, blood pressure, physiological parameters, and categorized trauma details for the dataset. The exact numbers were broken into (# available data/51) as the forthcoming analyses have different sample sizes due to missing data in some measures.

baseline and trauma/baseline data. For all correlation analyses, we adjusted for age, sex, height, weight, BMI, race, ethnicity, and education. 3) After carrying out signal processing steps in Figures 5.3 and 5.4, and steps in Figure 5.5, we constructed a machine learning dataset. For our main analysis, we excluded EDA and HRV features as they limited the dataset to a small number of patients as EDA signal was very noisy. Our dataset included time series features, patient background/demographics, and cuff-based blood pressure values (see Figure 5.3 for time series features, static features, and background/demographics coding). We created three datasets: one by using only baseline data (5-15 minutes of data/subject), one by using trauma/pre-trauma (3-4 minutes of data/subject) data, and one by using trauma/baseline data (6-16 minutes of data/subject).

From a practical standpoint, baseline or trauma/pre-trauma is the easiest to obtain, trauma/baseline data requires clean baseline data. Using a LOSO-CV cross validation paradigm, we attempted to predict the PTSD status for each dataset. We used Naïve Bayes, SVM with linear, sigmoid, RBF, and polynomial kernels, Random Forest, k-NN (n = 5), Logistic Regression, Multilayer Perceptron, Decision Trees, and Adaboost using Python's scikit-learn toolbox.

5.3 Results

5.3.1 Baseline Analysis

PTSD (n = 25) and non-PTSD (n = 26) groups are not significantly different in terms of age, sex, height, weight, BMI, education, age (Table 5.1, $p > 0.05$). For psychometric surveys, we observe that PTSD group has significantly higher PCL-C, PTSD-SS, HAM-A, HAM-D, CADSS, BDI, ETI, ATI scores, as expected due to the overlapping symptoms

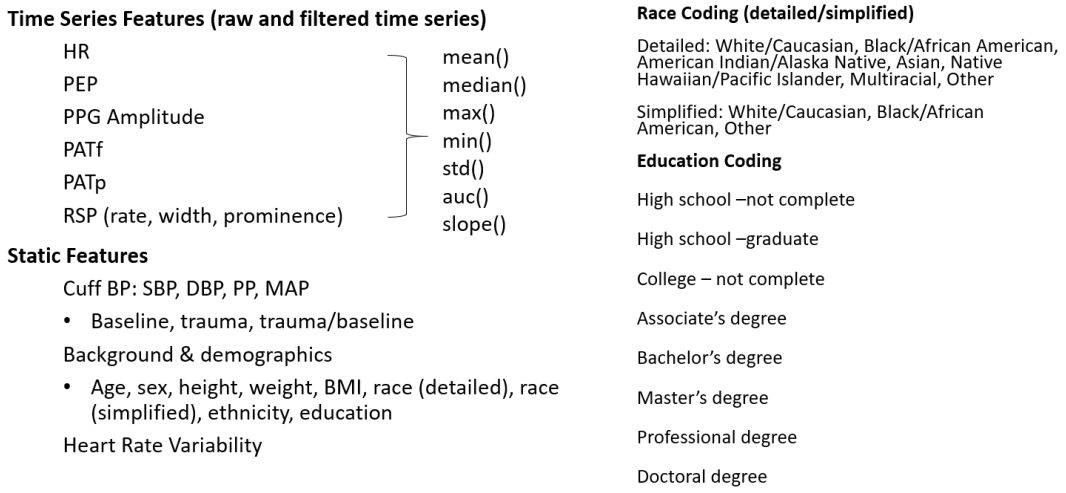

Figure 5-3. The list of features and relevant coding used in machine learning. Dataset includes both dynamic (time series) and static (BP, HRV, background demographics) features.

of PTSD with anxiety and depression, dissociative symptoms, and childhood/adulthood history of trauma ($p < 0.001$). The groups do not have a significant difference in ESSI scores. In terms of baseline personality surveys, the groups do not show significant difference in Big Five categories ($p > 0.05$), though notably non-PTSD group has less extraversion trait. When it comes to alexithymia (Table 5.2), PTSD group exhibits higher scores in difficulty in describing/identifying feelings and total alexithymia score ($p < 0.05$). From baseline mood scales, PTSD group experiences more distress, feels more nervous, anxious, and fearful, compared to the non-PTSD group ($p < 0.05$). Lastly, categorized traumas (onset, time, sexual content) do not seem to be significantly different between groups ($p > 0.05$).

5.3.2 Associations with Physiological Indices

We observe a number of significant associations between baseline and trauma/baseline physiological indices and clinician administered scales. Appendix Tables A.1 and A.2 list the statistically significant associations with correlation coefficients and p-values. After adjustments with potential confounders, a number of physiological measures are significantly correlated with psychometric scales, during both baseline and trauma (normalized by baseline).

For baseline, we see significant correlations between anxiety and depression scales and EDA measures, PTSD status/severities with PAT, respiratory measures, and HRV measures. Baseline HR is associated with Agreeableness and Openness personality traits, baseline EDA measures are associated with subjective discomfort, baseline respiratory and

HRV measures are associated with VISAN mood scales, and HRV measures are associated with the Big Five personality traits.

Table 5-1. PTSD and Non-PTSD Dataset Details - 1

PTSD Status	Demographics & Background					
	Age	**Sex**	**Height**	**Weight**	**BMI**	**Education**
PTSD	35.2 (12.6)	19F, 6M	170.1 (10.6)	83.3 (24.6)	28.4 (6.2)	4 (1)
Non-PTSD	30.7 (9.6)	15F, 12M	170.2 (10.7)	77.9 (13.7)	27 (4.9)	4.6 (1.3)
P-value	0.16	0.13	0.99	0.33	0.39	0.1

	Psychometric Scales									
	PCL-C	**CAPS**	**PTSD-SS**	**HAM-A**	**HAM-D**	**CADSS**	**BDI**	**ETI**	**ATI**	**ESSI**
PTSD	11.9 (13.8)	9 (10.5)	7.9 (9.5)	7.6 (91)	8.3 (11.1)	7.8 (8.6)	13.1 (14.9)	10.4 (12.3)	2.4 (2.9)	5.4 (6.6)
Non-PTSD	7.9 (9.3)	N/A	3.4 (4.3)	4.8 (6.8)	4.6 (6.4)	2.2 (3.5)	8.6 (10.5)	5.2 (6.8)	1.8 (2.1)	5.5 (6.7)
P-value	<0.001	N/A	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.18

	Big Five Personality Scale				
	Extraversion	**Agreeableness**	**Conscientiousness**	**Neuroticism**	**Openness**
PTSD	20.9 (6.2)	26.9 (5.6)	28.9 (6.9)	24.4 (6)	35.2 (7.2)
Non-PTSD	30.5 (3.5)	35 (2)	30.5 (3.5)	22 (1)	31.5 (4.5)
P-value	0.06	0.08	0.76	0.61	0.52

Datasets do not differ in terms of the analyzed demographics and background (race not shown as race coding do not relate to any quantifiable severity). Patients with PTSD exhibit higher severities in psychometric scales. BMI = Body mass index, F = Female, M = Male. Values represented as mean ± SD.

Table 5-2. – PTSD and Non-PTSD Dataset Details - 2

PTSD Status	Toronto Alexithymia Scale (TAS)				
	Difficulty in Describing Feelings	**Difficulty in Identifying Feelings**	**Externally Oriented Thinking**	**TAS Score**	**Alexithymia Class**
PTSD	15.5 [1]	20.4 (5.7)	16.9 (3.6)	52.7 (12.5)	0.9 (0.9)
Non-PTSD	5.5 (0.5)	10 (2)	13.5 (1.5)	29 (4)	0 (0)
P-value	0.02	0.03	0.25	0.03	0.19

	SUDS	**VISAN**				
	Discomfort	**Nervous**	**Anxious**	**Fearful**	**High**	**Anger**
PTSD	33.5 (27.9)	2 (1.5)	2.2 (1.5)	1.1 (1.2)	0.3 (0.6)	0.4 (0.9)
Non-PTSD	12.6 (15.2)	0.4 (0.7)	0.4 (0.7)	0 (0.2)	0.2 (0.6)	0 (0.2)
P-value	<0.01	<0.0001	<0.0001	<0.0001	0.66	0.09

	Trauma Details		
	Onset (>6 months?)	**Time (Childhood/Adulthood)**	**Sexual Content (Y/N)**
P-value	0.28	0.5	0.23

Patients with PTSD exhibit higher scores in alexithymia categories, higher baseline discomfort, nervousness, anxiety, and fear. There is no significant difference between groups in terms of trauma onset, time, and sexual content. SUDS = Subjective Units of Distress, VISAN = Visual Analogue Scale. Values represented as mean ± SD.

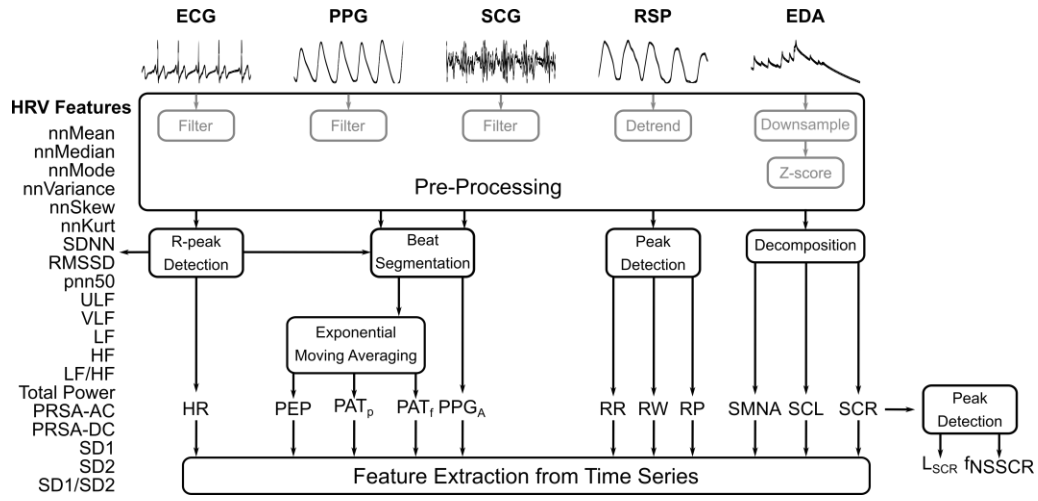

Figure 5-4. Signal processing for time series features and static HRV features.

For trauma reactivity (trauma/baseline), we see associations between HR/ PPG Amplitude and CAPS/ETI, EDA measures and anxiety, depression, social support. Blood pressure reactivity is strongly correlated with alexithymia symptoms, and HRV measures are associated with self-reported mood ratings, anxiety, and depression scales.

5.3.3 Classifier Performance and Feature Analysis

Table 5.3 shows the ROC AUC and selected machine learning results. Using baseline dataset, we obtain a ROC AUC of 0.62, little above naïve prediction. Normalized trauma (baseline and pre-trauma) datasets perform notable higher in the classification task. Trauma/baseline dataset gives a ROC area of 0.98. However, as using pre-trauma data would be more practical from an engineering standpoint, we focus on the trauma/pretrauma dataset. We observe that SVM with sigmoid kernel gives the highest ROC of 0.89. The accuracies for these datasets range from 78% to 98%.

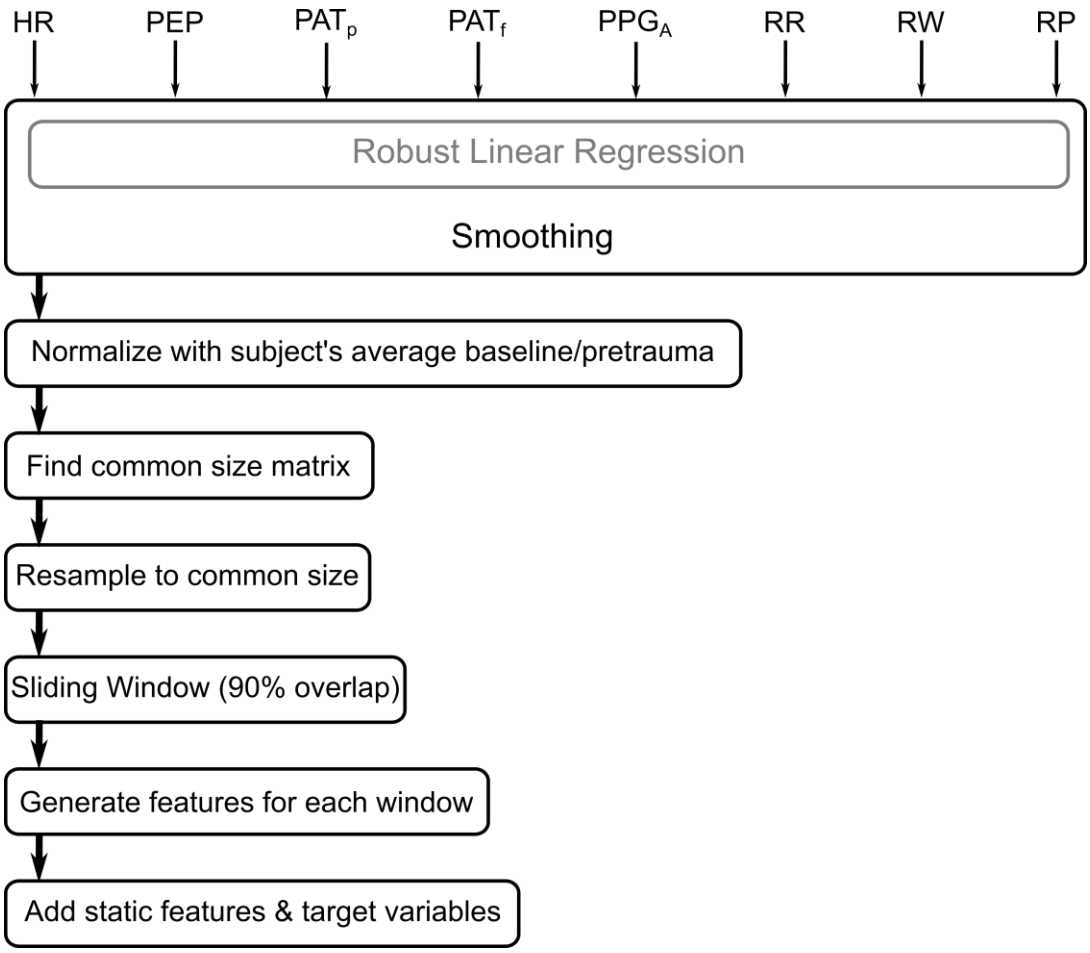

Figure 5-5. Dataset construction for time series features (except EDA as it was excluded due to low sample size).

Next, we investigate the relative importance of features to determine the contribution of different feature types and sensors. As sigmoid boundary is non-linear, this is not a trivial task. We categorized the features and dropped one by one to understand the drop in ROC, hence their relative contribution. The features were categorized as the following groups: demographics & age, BP (all cuff-based BP values), HR, PEP, PPG Amplitude, PAT (foot, peak), respiratory features (RR, RW, RP). Table 5.4 shows the ROC areas when only one of the feature groups is used in machine learning with the same classifier. The highest

Table 5-3. Machine Learning Results

	Trauma/Baseline Dataset				
	F-1 Score	**Precision**	**Recall**	**# Subjects**	**ROC AUC**
Non-PTSD	0.98	0.95	1	23	0.98
PTSD	0.98	1	0.96	23	
Accuracy	0.98	0.98	0.98		
Macro Avg.	0.98	0.98	0.98	46	
	Trauma/Pretrauma Dataset				
Non-PTSD	0.79	0.79	0.79	23	0.89
PTSD	0.79	0.79	0.79	23	
Accuracy	0.79	0.79	0.79		
Macro Avg.	0.79	0.79	0.79	46	

ROC AUC occur when HR and PEP were used separately, PAT features result in the lowest ROC AUC.

5.4 Discussion and Conclusion

We performed a wide scale assessment using multimodal autonomic nervous system activity, background/demographics, and clinician administered psychometric surveys. Our results reveal that reactivity-based PTSD assessment holds promise, compared to using only baseline data. The methods presented herein could be used in objective assessment of

Table 5-4. ROC AUC for Individual Feature Groups (Trauma/Pretrauma Dataset)

	Demographics	**BP**	**HR**	**PEP**	**PPG Amp.**	**PAT**	**RSP**
ROC AUC Drop	0.81	0.55	0.96	0.97	0.70	0.5	0.80

PTSD and could complement the existing interview-based methodologies. The use of wearable sensing data and minimum requirement for a medical professional potentially widens the applicability to disadvantaged populations who would otherwise not have the chance to visit a clinic.

Confirming relevant literature [50], HRV values are significantly associated with clinical assessments of anxiety, depression, and PTSD. Autonomic imbalance in PTSD has started to be recognized in the recent years [6]. Recent studies including our group is contributing to the literature linking the autonomic nervous system problems to PTSD, depression, and heart disease [375-377].

This is the first study, to our knowledge, to use multimodal sensing during a traumatic recall paradigm to identify PTSD with a dataset that includes females. PTSD classification have been studied previously with emotionally evocative virtual reality [367] and speech data during PTSD-related structured interview [368] with only male participants. These studies significantly differ from the current study due to only male datasets, experiment protocols, and sensing modalities. [367] investigated only HR and EDA differentiation during a virtual reality based emotional paradigm in male veterans. [368] studied speech features during a CAPS interview from male participants. Given that women are more

likely to develop PTSD than men, the inclusion of women in the dataset is important for the potential of the work to successfully translate from lab to patient care settings.

Alexithymia and mood survey results are quite interesting: Patients with PTSD exhibit great difficulty in describing and identifying feelings with a higher total alexithymia score, at the same time they report higher levels of baseline discomfort, nervousness, anxiety, and fear. The link between PTSD and alexithymia have been investigated by a few groups [378-381]. Alexithymia in PTSD have been thought as a characteristic related to PTSD symptoms, and not simply exposure to trauma [378], as a measure of suppressed or warded-off negative effects. Associations between PTSD severity and dysfunctional brain regions in PTSD also point out the merit of alexithymia measurement [380]. Despite the difficulties in expression of feelings, higher self-reported negative mood scores highlight a paradoxical outcome.

A notable point to mention is the type of autonomic perturbation. One might think that stressors not specific to trauma (i.e., mental stress, exercise) could potentially be helpful in PTSD assessment. Research showed that reactivity to non-personalized emotional cues or stressors is comparable or slightly greater in magnitude in patients with PTSD than in control groups [364]. Specifically, prior studies show that psychophysiological reactivity to emotional cues not related to trauma was only able to accurately classify 46% of individuals who met diagnostic criteria for PTSD and 76% of individuals who did not meet diagnostic criteria for PTSD. These sensitivity and specificity values are significantly lower than our results obtained with personalized traumatic scripts. Nevertheless, the method for delivery of trauma-related cues (audible, imagery, thought, only trauma-specific cues such as smell or horns) is debatable and should be researched.

Another point is the ethical and practical consideration of using traumatic event recollections. The requirement to use traumatic events is a baseline condition for clinician administered surveys based on DSM-5 [39, 40]. These surveys ask questions about the traumatic event details and posttraumatic symptoms. Moreover, prolonged exposure therapy, which is an intervention strategy for patients with PTSD, uses traumatic event recollections and trauma-related cues to gradually improve symptoms of PTSD [364]. Hence, the use of traumatic memories is quite common in both diagnosis and treatment of PTSD, though with longer amounts of time and the requirement to interact with a medical professional. Our study uses one to two minutes of traumatic stress scripts, substantially decreasing the required amount of time and passively listening voice recordings.

This study is subject to limitations: Due to the small sample size and lack of high quality EDA signal, the merit of EDA and HRV (two of the most commonly used psychophysiological indices) cannot be evaluated for the classification task with the same sample size. Though the partial correlation assessments signal the promising merit of these parameters, it was left as future work to evaluate with lower (available) sample sizes and compare with other physiological parameters.

In summary, we were able to classify PTSD status based on autonomic reactivity to traumatic event recollections, as captured by signals that can be measured with wearable technology. This assessment might potentially complement standard PTSD measures by providing objective, real-world assessments that could enhance clinical and non-clinical care with the help of wearable sensing devices. We believe that our autonomic markers represent a multidimensional set of “internet of body” features which hold promise by further validation for developing objective, low-cost, and non-invasive PTSD diagnosis.

Given the ubiquity of smartphones and small form factor physiological sensing modalities [207, 382, 383], the objective assessment could be a widely accessible tool for assessing PTSD in civilian, veteran, or military populations.

CHAPTER 6. CONCLUSION AND FUTURE WORK

6.1 Conclusion

PTSD is a debilitating disorder with profound public health burden. Individuals with PTSD face high levels of disability and loss of productivity. With the COVID-19 pandemic, we have once again been reminded of the importance of healthcare accessibility, regardless of socioeconomic status. Unfortunate (though predicted) events such as COVID-19 signal the human psychology that "anything can happen any time", thus leading to vigilance, alertness, and sustained attention. Such alertness and stressed state are not sustainable for the human body, and eventually will lead to mental and physical health problems. Wearable bioelectronic medicine and digital phenotyping have the potential for revolutionizing healthcare, specifically mental healthcare, for those who cannot afford or maintain continuous interaction with clinicians and caregivers.

Psychiatry does not have the lab work that is common in other medical disciplines, such as readily-available blood biomarkers. It relies on subjective, self-reported measures, and can be unreliable because of human factors. The current diagnostic tools are not perfectly aligned with the neurobiology of psychiatric disorders. This alignment could be addressed with engineering, by connecting dots between current diagnostics and neurobiology with the aim of defining objectively measurable quantities relevant to disease-specific circuits.

In this dissertation, we investigated techniques to diagnose and treat PTSD. We first developed technologies in the context of stress measurement and autonomic nervous system activity quantification. We then validated the potential of noninvasive VNS with

investigations in noninvasive sensing and blood biomarkers that are relevant to the condition, in both PTSD and non-PTSD controls with a sham-controlled double blind study. We demonstrated that pairing noninvasive VNS with traumatic reminders in patients with PTSD reduces the fight-or-flight response to emotional triggers and thereby could provide a completely new mode of treatment. This paradigm also reduces stress reactivity to non-personalized mental stress and stress reactivity in trauma-exposed individuals without PTSD. We showed that capturing autonomic nervous system activity could be useful to quantify meaningful information about stimulation and disease state. We developed a machine learning-guided target engagement quantification method based on fusing multiple modalities that can be incorporated in wearable technology. Lastly, we developed an autonomic reactivity based objective PTSD assessment scheme that predicts the PTSD status using traumatic memories, for which the state-of-the-art diagnosis methods rely on clinician administered structured interviews and self-reports. In sum, this work established methods amenable for widespread use of noninvasive VNS in the context of stress and PTSD.

6.2 Future Work

Various future research directions could stem from this work. A potentially domain-specific direction would be application to other disorders that VNS could be useful for, such as opioid use disorder, anxiety disorders, obsessive compulsive disorder, or mild traumatic brain injury occasionally seen together with PTSD. These disorders have overlapping symptoms and neurobiology with PTSD, thus could benefit from the advantages of noninvasive neuromodulation. Peripheral neuromodulation projects to a set

of brain regions that regulate functions from memory to sleep, thus providing many application areas.

A more computationally focused direction is personalization of stimulation based on a patient's individual response. Characterization and personalization of neuromodulation treatments are crucial as each patient is substantially different from another. Personalized modeling and control of neuromodulation-related biomarkers will pave the way for widespread adoption of these technologies. Improvement of stimulation selectivity and parameter studies are also natural research extensions. Cost-effective and portable modalities are required to research selectivity and optimization of stimulation parameters based on the target domain.

6.2.1 Ethical Considerations

Although the methodologies presented in this dissertation are solely for academic investigation, it is important to discuss ethical considerations for multimodal physiological monitoring and physiological modulation, particularly in the context of affective computing (i.e., psychiatric disease prediction, emotion recognition) or mood/performance improvement (i.e., enhancing resilience). Physiological data can be very personal and revealing. Though the researcher/engineer designs these systems with enormous creativity, the fundamental ethical side of tracking and modulating human emotions and emotional phenotyping in different sectors should be taken into consideration before turning these technologies into actual products or services, or sharing research content publicly.

Privacy-Aware Technologies and Research Activities

Particularly for businesses handling big data, encryption of physiological or digital data in ways that would not be trivially tracked backwards by robust security & cryptography algorithms not to reveal identity is crucial. Asides from big businesses, data, code, and model sharing is quite common in research domain for relatively small datasets. Research sharing could be customized in a way that would not be decoded as individualized information, rather as average/generalized outputs. For instance, rather than public availability of machine learning models trained to output specific personal information, the models could only produce an average/general representation of the output. In addition, research datasets could suffer from biases due to uneven distribution of demographics, race, education level, or cultural background. These limitations should be communicated with the model user for careful testing with new data.

Fully Informed Consent and Ability to Opt-Out Anytime

The data belongs to the user or the subject under experimentation, regardless of the aim of the product of service. It must be the data owner's decision as to how much they want to share and whether they want to opt-out to remove their entries from the entire data ecosystem. Consent process requires clear communication by avoiding unnecessary technical language and lengthy documentation.

Transparent Tech and Data Storage

It is quite important to inform the naïve user about the specifics of data handling and storage, and precautions taken against potential pitfalls. *Where is the data stored? What*

kind of privacy protocols are followed? Who accesses the data? Is the data de-identified? Regardless of the technical knowledge of the data owner, critical details about data handling and storage should be communicated in a language the data owner can understand and evaluate for themselves.

To this end, it is at the designer's discretion and very personal to the designer to follow or not follow any sort of morality or ethical considerations while moving forward with a particular task or producing products aimed at benefiting consumer welfare. For what it's worth, while building emotional connections with technology, one needs to keep in mind that *great power comes with great responsibility*.

Neuromodulation or "electroceuticals" may not completely replace pharmaceuticals, however there is great promise that they can complement them and reduce the burden on drug side effects and pharmaceutical-resistant conditions. Wearable technologies can substantially help to achieve these goals. While designing these technologies, one should be considerate on the usability, security, privacy, and ethical aspects. Wearable and ubiquitous sensing provides us a wide range of tools where we could benefit from multiple streams of data, different measurement techniques, and computational methods to make sense of the data. The nexus of such technological advancements could lead to wearable sensing feedback enabled neuromodulation therapies that can transform the diagnosis and management of neuropsychiatric disorders.

LIST OF PH.D. TRAINING PUBLICATIONS

Peer-Reviewed Journal Publications

[J15] **NZ Gurel**, H Jung, MT Wittbrodt, SL Ladd, AJ Shah, V Vaccarino, JD Bremner, OT Inan, "Automatic Detection of Target Engagement in Transcutaneous Cervical Vagal Nerve Stimulation for Traumatic Stress Triggers", *IEEE Journal of Biomedical and Health Informatics (IEEE JBHI)*, 24 (7), 1917-1925, 2020.

[J14] **NZ Gurel**, M Huang, MT Wittbrodt, H Jung, SL Ladd, MH Shandhi, YA Ko, L Shallenberger, JA Nye, BD Pearce, V Vaccarino, AJ Shah, JD Bremner, OT Inan, "Quantifying Acute Physiological Biomarkers of Transcutaneous Cervical Vagal Nerve Stimulation in the Context of Psychological Stress", *Brain Stimulation*, 13 (1): 47-59, 2019.

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[J10] AH Gazi, **NZ Gurel**, KL Scott, MT Wittbrodt, AJ Shah, V Vaccarino, JD Bremner, OT Inan, "Investigating Digital Cardiovascular Biomarker Responses to Transcutaneous Cervical Vagus Nerve Stimulation: State-Space Modeling, Prediction, and Simulation", *in press, JMIR mHealth and uHealth*, 2020.

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Journal Manuscripts (Under Review)

[J7] **NZ Gurel**, MT Wittbrodt, H Jung, MH Shandhi, EG Driggers, SL Ladd, M Huang, YA Ko, L Shallenberger, JA Nye, BD Pearce, V Vaccarino, AJ Shah, OT Inan, JD Bremner, "Transcutaneous Cervical Vagal Nerve Stimulation Blunts Sympathetic Responses in Posttraumatic Stress Disorder", *under review*, 2020.

[J6] **NZ Gurel***, Y Jiao*, MT Wittbrodt, YA Ko, A Hankus, SL Ladd, L Shallenberger, N Murrah, M Huang, A Haffer, J Alkhalaf, O Levantsevych, JA Nye, V Vaccarino, AJ Shah, OT Inan, BD Pearce, JD Bremner, "Transcutaneous Cervical Vagus Nerve Stimulation Blocks Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Response to Stress: A Randomized, Sham Controlled, Double Blind Investigation ", *under review*, 2020. [*co-first authors].

[J5] JD Bremner, **NZ Gurel**, Y Jiao, MT Wittbrodt, O Levantsevych, M Huang, H Jung, MH Shandhi, J Beckwith, I Herring, MH Rapaport, N Murrah, EG Driggers, YA Ko, J Alkhalaf, M Soudan, J Song, B Ko, L Shallenberger, A Hankus, JA Nye, J Park, V Vaccarino, AJ Shah, OT Inan, BD Pearce, "Transcutaneous Vagal Nerve Stimulation Blocks Stress-Induced Activation of Interleukin-6 and Interferon-γ in Posttraumatic Stress Disorder: A Double-Blind, Randomized, Sham-Controlled Trial", *under review*, 2020.

[J4] JD Bremner, **NZ Gurel**, MT Wittbrodt, MH Shandhi, MH Rapaport, JA Nye, BD Pearce, V Vaccarino, AJ Shah, J Park, M Bikson, OT Inan, "Application of Noninvasive Vagal Nerve Stimulation to Stress-Related Psychiatric Disorders", *under review*, 2020.

Journal Manuscripts (In Preparation)

[J3] **NZ Gurel**, A Gazi, J Hernandez, MT Wittbrodt, H Jung, V Vaccarino, AJ Shah, BD Pearce, JD Bremner, OT Inan, *in preparation*, 2020.

[J2] MT Wittbrodt, **NZ Gurel**, H Jung, MH Shandhi, V Vaccarino, AJ Shah, BD Pearce, JD Bremner, OT Inan, *in preparation*, 2020.

[J1] SE Sheikh, **NZ Gurel**, G Clifford, OT Inan, AJ Shah, *in preparation*, 2020.

Book Chapters

[B2] MT Wittbrodt, **NZ Gurel**, PK Mehta OT Inan, JD Bremner, "Noninvasive Vagus Nerve Stimulation in Posttraumatic Stress Disorder", *submitted,* 2020

[B1] JD Bremner, MT Wittbrodt, **NZ Gurel**, MH Shandhi, AH Gazi, J Park, OT Inan, "Transcutaneous Vagal Nerve Stimulation in Trauma Spectrum Disorders", In *Vagal Nerve Stimulation* by Eric Porges and Martin Frasch, *submitted,* 2020

Peer-Reviewed Conference Publications, Abstracts, Live Demos

[C12] **NZ Gurel***, AH Gazi*, KL Scott, MT Wittbrodt, AJ Shah, V Vaccarino, JD Bremner, OT Inan, "Timing Considerations for Noninvasive Vagal Nerve Stimulation in Clinical Studies", *American Medical Informatics Association Annual Symposium (AMIA '19),* Washington, DC, 2019. [oral presentation, 10-pages, session offering MOC-II credit to practicing clinical informaticians, *co-first authors]

[C11] **NZ Gurel**, MT Wittbrodt, AJ Shah, V Vaccarino, OT Inan, JD Bremner, "Noninvasive Vagal Nerve Stimulation Effects on Anger Response", *IEEE Conference on Biomedical Health Informatics (IEEE BSN/BHI '19),* Chicago, IL, 2019. [poster, 1-page]

[C10] AH Gazi, **NZ Gurel**, KL Scott, MT Wittbrodt, AJ Shah, V Vaccarino, JD Bremner, OT Inan, "Preliminary Modeling of the Kinetics of Photoplethysmogram Changes Following Noninvasive Vagus Nerve Stimulation", *IEEE Conference on Biomedical Health Informatics (IEEE BSN/BHI '19),* Chicago, IL, 2019 [poster, 1-page] **[Best Poster Award, 2nd Place]**

[C9] Y Jiao, YA Ko, **NZ Gurel**, A Hankus, SL Ladd, MT Wittbrodt, L Shallenberger, N Murrah, M Huang, A Haffer, J Alkhalaf, H Jung, O Levantsevych, JA Nye, MH Shandhi, V Vaccarino, AJ Shah, OT Inan, JD Bremner, BD Pearce, "Levels of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in Posttraumatic Stress Disorder and Modulatory Effect of Noninvasive Cervical Vagus Nerve Stimulation", *Society for Neuroscience (SfN '19),* Chicago, IL, 2019 [poster, 1-page]

[C8] **NZ Gurel**, MH Shandhi, JD Bremner, V Vaccarino, SL Ladd, L Shallenberger, AJ Shah, OT Inan, "Toward Closed-loop Transcutaneous Vagus Nerve Stimulation Using Peripheral Cardiovascular Physiological Biomarkers: A Proof-of-concept Study", *IEEE Conference on Wearable and Implantable Body Sensor Networks (IEEE BSN/BHI '18),* Las Vegas, NV, 2018. [oral presentation, 4-pages] **[Best Paper Award, 2nd Place]**

[C7] **NZ Gurel**, H Jeong, HE Kloefkorn, S Hochman, OT Inan, "Unobtrusive Heartbeat Detection from Mice Using Sensors Embedded in the Nest*", IEEE Engineering in Medicine and Biology Conference (IEEE EMBC '18),* Honolulu, HI, 2018. [oral presentation, 4-pages] **[Best Paper Finalist Award]**

[C6] **NZ Gurel***, D Ward*, FL Hammond, OT Inan, "Live Demonstration: A Soft Thermal Modulation System with Embedded Fluid Channels for Neuro-Vascular Assessment", *IEEE Biomedical Circuits and Systems Conference (IEEE BioCAS '18)*, Cleveland, OH, 2018 [live demo, 1-page, *co-first authors]

[C5] H Jeong, **NZ Gurel**, HE Kloefkorn, S Hochman, OT Inan, "Performance of Unobtrusive Detection of High Frequency Heart Rate Variability in Mice Using an

Instrumented Nest", *IEEE Life Sciences Conference (IEEE LSC '18),* Montreal, Canada, 2018 [oral presentation, 4-pages]

[C4] D Ward*, **NZ Gurel***, OT Inan, FL Hammond, "A Soft Thermal Modulation and Physiological Sensing System for NeuroVascular Assessment", *IEEE Conference on Robotics and Biomimetics (IEEE ROBIO '18),* Kuala Lumpur, Malaysia, 2018 [oral presentation, 8-pages, *co-first authors]

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APPENDIX A. SUPPLEMENTARY MATERIAL

Table 0-1. Statistically significant associations ($p<0.05$) during baseline for PTSD and Non-PTSD dataset.

Physiological Measure	Psychometric Scales and Correlation Coefficients	Physiological Measure	Psychometric Scales and Correlation Coefficients
HR	Agreeableness (Big Five): -0.72 Openness (Big Five): -0.84	**pnn50**	Agreeableness (Big Five): 0.82 Alexithymia Status (TAS): -0.97
PAT	PTSD Status: 0.33	**ULF**	Difficulty Describing Feelings (TAS): -0.98 Total Alexithymia Score (TAS): -0.96
SCL_{MEAN}	HAM-A: -0.64 HAM-D: -0.60 SUDS: -0.71	**VLF**	HAM-A: -0.34
SCR_{MEAN}	HAM-A: 0.64 HAM-D: 0.60 SUDS: 0.71	**LF**	PTSD Status: -0.33
SMNA	HAM-D: 0.6 SUDS: 0.71	**HF**	Extraversion (Big Five): 0.88 Neuroticism (Big Five): -0.86 Openness (Big Five): 0.96
SCL_{SLOPE}	ESSI: -0.65	**LF/HF**	Conscientiousness (Big Five): -0.81
RR	PTSD-SS: -0.38	**Total Power**	Extraversion (Big Five): 0.81
RW	PTSD-SS: 0.41 PTSD Status: 0.31 Highness (VISAN): 0.33 Anger (VISAN): 0.33	**NNkurt**	Nervousness (VISAN): -0.36 Anxiousness (VISAN): -0.36

NNmean	Neuroticism (Big Five): -0.81 Openness (Big Five): 0.84	**AC**	Openness (Big Five): -0.91
NNmedian	Neuroticism (Big Five): -0.80 Openness (Big Five): 0.83	**DC**	Openness (Big Five): 0.92
NNskew	Neuroticism (Big Five): -0.92 Openness (Big Five): 0.81	**SD1**	Extraversion (Big Five): 0.91 Neuroticism (Big Five): -0.88 Openness (Big Five): 0.90
NNiqr	Extraversion (Big Five): 0.89	**SD2**	HAM-A: -0.36 HAM-D: -0.39 Extraversion (Big Five): 0.91
SDNN	HAM-D: -0.36 Extraversion (Big Five): 0.93	**RMSSD**	Extraversion (Big Five): 0.91 Openness (Big Five): 0.90

Table 0-2. Statistically significant associations ($p < 0.05$) for trauma/baseline for PTSD and Non-PTSD dataset

Physiological Measure	Psychometric Scales and Correlation Coefficients	Physiological Measure	Psychometric Scales and Correlation Coefficients
DBP	Conscientiousness (Big Five): -0.82 Externally Oriented Thinking (TAS): 0.82 Total Alexithymia Score (TAS): 0.81	**LF/HF**	PCL-C: 0.44 PTSD Status: 0.4 HAM-A: 0.35 CADSS: 0.40 ETI: 0.47 ATI: 0.43 Neuroticism (Big Five): -0.91 Openness (Big Five): 0.83 Anxious (VISAN): 0.39
MAP	Conscientiousness (Big Five): -0.79	**AC**	CAPS: -0.75 Neuroticism (Big Five): 0.89 Openness (Big Five): -0.88 Anger (VISAN): 0.39
PP	Extraversion (Big Five): 0.80 Alexithymia Status (TAS): -0.98	**DC**	Openness (Big Five): -0.83
HR	CAPS: 0.64 ETI: 0.39 Extraversion (Big Five): 0.81	**RR**	PTSD-SS: 0.34 CADSS: 0.34 BDI: 0.33
PPG Amplitude	ETI: 0.55 ATI: 0.43	**RW**	CADSS: -0.32 BDI: -0.33
SCL_{MEAN}	ESSI: -0.66	**RP**	PTSD-SS: 0.35

			HAM-A: 0.39 HAM-D: 0.39 CADSS: 0.42 BDI: 0.31 Nervousness (VISAN): 0.38 Anxiousness (VISAN): 0.40 Fear (VISAN): 0.37 Highness (VISAN): 0.40 Anger (VISAN): 0.71
SCR_{MEAN}	ESSI: -0.66	**NNvariance**	Anger (VISAN): 0.44
SMNA	ESSI: -0.66	**LF**	CAPS: -0.70

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