A Translational Investigation of the Effect of Genital and Tibial Neuromodulation on Gynecological Hemodynamics

by

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Dedication

This dissertation is dedicated to my mother, Trang Doan Nguyen. She is the strongest and most selfless person I know. I owe it all to you ma. I love you.

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Elizabeth C. Bottorff

Table of Contents

List of Tables

List of Figures

Abstract

Sexual function is intricately tied to life satisfaction and has an important role in social, emotional, and mental well-being. An estimated 22-43% of women have poor sexual function, and people with spinal cord injury (SCI) also report it as a high priority to restore. Existing treatment options are limited, particularly for the broad range of gynecological dysfunctions. Neuromodulation has the potential to treat sexual dysfunction by providing a targeted therapy that taps into existing neural circuits. Pudendal nerve and tibial nerve neuromodulation have demonstrated potential as a treatment in preclinical and clinical studies, but more needs to be understood about their mechanisms for improving female sexual dysfunction (FSD) symptoms. In this dissertation, I performed translational studies examining physiological responses to neuromodulation in rodent and human subjects under different paradigms that yielded insights into its potential as a therapy for sexual dysfunction.

The first Aim of my dissertation was to investigate the effect of pudendal and tibial nerve stimulation on genital blood flow in anesthetized rats. Vulvar blood perfusion was recorded with a laser speckle contrast imager during baseline, nerve stimulation, and recovery periods. I found that pudendal, but not tibial, nerve stimulation can increase vulvar blood perfusion during stimulation. These results suggest that pudendal stimulation can drive short-term responses and that rodent vulvar blood perfusion needs further investigation to determine its potential as a preclinical sexual function biomarker.

In the second Aim of this dissertation, we measured genital blood flow with vaginal photoplethysmography (VPG) during baseline, stimulation, and recovery periods for genital

(distal pudendal nerve) and tibial nerve stimulation (GNS/TNS). We recruited three cohorts of participants in a randomized crossover study design: women with SCI, participants with FSD, and non-dysfunction, healthy controls. We observed variable changes in genital arousal across participants and found that GNS but not TNS can increase subjective arousal, including in SCI participants. I hypothesize that differences between animal and human anatomy and cortical arousal states were contributing factors to the dissimilarity in genital arousal responses between the first two Aims. These factors are important to consider in future translational studies.

A primary reason I believe the physiological data for Aim 2 were inconclusive was due to descending cortical inhibition and a lack of sexual stimuli in the study protocol. This observation led to my third dissertation Aim, in which we investigated the effect of GNS and TNS on the VPG response to erotic stimuli in the same cohorts as Aim 2. Participants viewed a sequence of neutral and erotic videos without and with nerve stimulation. We found low sexual concordance between VPG and subjective arousal and no conclusive trends in physiological genital arousal across all three groups. As in Aim 2, SCI and FSD participants expressed willingness to continue using neuromodulation. This study reaffirms the need to identify better biomarkers for sexual function that correlate with the sexual experience of people with gynecological anatomy.

This dissertation examined neural control over gynecological arousal responses in rodent and human subjects and identified key areas of improvement for translational research in female sexual medicine. Although physiological responses varied within and across these studies, SCI and FSD study participants consistently expressed an interest in neuromodulation as a treatment for a condition that significantly affects their lives. This research further underscores the impact of FSD and the need for improved treatments.

Chapter 1 Introduction

Sexual function is an essential part of life and has been linked to physical, mental, and social well-being as well as overall life satisfaction[1]. It is especially important to women with neurological damage, as they report sexual function one of their top priorities to regain after injury[2,3]. Limited treatment options and healthcare disparities call for more research into the etiology and classifications of female sexual dysfunction (FSD)[4]. Reviews on the neurophysiology of female sexual function within the last few decades focus on the central nervous system[1,2] , leaving the role of the peripheral nervous system (PNS) relatively unexplored. For example, only in 2019 did researchers perform an anatomical and histological analysis of the clitoris, providing valuable insights for vulvar surgeries[5]. However, technological advancements in neural engineering have opened a unique opportunity to study the role of peripheral nerves in female sexual function. In this thesis, I discuss two potential non-invasive neuromodulation therapies: genital nerve stimulation (GNS) and tibial nerve stimulation (TNS). I hypothesize that both therapies modulate genital arousal, a critical component of healthy sexual function, albeit through different mechanisms. Then, I share the animal and human subjects research I have done that contribute to our understanding of the peripheral circuitry and spinal reflexes that control sexual arousal in people with vulvas. Finally, I discuss the findings from this dissertation and suggested directions for this research and intersectional considerations.

1.1 Neurophysiology of Female Sexual Arousal

Sexual function research, historically skewed towards a male or reproductive lens, only recognized female sexual function as a distinct field of research with the publication of the widely controversial and influential scholarly book, "Sexual Behavior in the Human Female" in 1953[6]. One aspect of sexual function that is especially dimorphic is the sex response cycle. Genital arousal is a critical component of the female sexual response cycle because it is necessary to produce lubrication among other physiological changes that are necessary for a satisfying sexual experience. This section will discuss the role of genital arousal in sexual function, the sex response cycle, how impaired genital arousal can contribute to female sexual dysfunction, and the underlying neurophysiology.

1.1.1 Genital Arousal and the Sex Response Cycle

The most well accepted model for sexual function across all sexes is the sexual tipping point or the dual control theory, but it is worth noting that it was initially developed for male erectile dysfunction. In this model, there are a variety of biological (e.g., hormones), psychological (e.g., mental health), and social (e.g., relationship status) factors that push an individual towards or away from a state of sexual receptivity. It is well established that factors in one area can influence others[7]. For example, a biological aspect of a sexual encounter, such as lubrication, may occur as desired but if the overall encounter is dissatisfying it may not re-enforce emotional intimacy and lead to decreased libido. Unfortunately, the lack of reliable and clinically meaningful female genital blood flow measurements is one of the main obstacles to better understanding the interactions between the different aspects of the sexual response cycle for people with gynecological anatomy. This chapter and thesis will focus on biological control of female genital blood flow specifically neurophysiology.

Given a healthy person, genital arousal, lubrication, and orgasm occur in response to acceptable sexual stimuli, as determined by the individual, and contribute to a satisfactory sexual experience. In people with gynecological anatomy, genital arousal is characterized by an increase in blood flow to the genitals that causes relaxation of the smooth muscle, engorgement of the clitoris, vasocongestion of the vaginal vestibule, and lubrication that is necessary to facilitate painless intercourse[8]. Genital arousal is accompanied by other physical changes such as an increase in heart rate, blood pressure, and respiration as well as increased sensitivity to auxiliary sex organs. Genital arousal can contribute to, but is not necessary for, the feeling of subjective arousal which increases the desire to seek out additional sexual stimuli creating a positive feedback loop until a resolution is reached[8]. Subjective arousal can be described as the cognitive engagement that occurs during interactions with sexual stimuli, usually measured by self-report questionnaires. The agreement of subjective and genital arousal is termed sexual concordance or synchrony and is typically lower in women than men but the significance of this is not understood[9]. Oftentimes that resolution is an orgasm, and although characterizations of the female orgasm remain a topic of debate, most definitions agree that it includes coordinated contractions of smooth and striated pelvic muscles, an end to the myotonia that contributes to vasocongestion, and an intense feeling of pleasure[10–12]. It is worth noting that a satisfying sexual experience does not always require an orgasm to occur, especially in people with vulvas[9]. In circumstances when orgasm does occur, it can be considered the peak of genital arousal since it is followed by a decrease in vasocongestion and at times, ejaculation.

1.1.2 Neural control of sexual arousal

Most of what we know about the central nervous system (CNS) mechanisms that control female sexual function are primarily focused on sexual behavior and motivation. These brain

regions include the ventral tegmental area, amygdala, septal region, prefrontal cortex, and cingulate cortex[13]. Human research on the brain regions that control genital arousal and orgasm are comparatively lacking[14]. The few human studies evaluating the correlation of brain activity and genital blood flow found that there was no significant correlation of the vaginal photoplethysmography measurements, the most common device used to measure genital blood flow, and fMRI scans in women with no history of sexual dysfunction[15,16]. The brain regions involved in genital arousal or orgasm identified via other methodology (e.g. electrical stimulation or lesion studies) are the thalamus, hypothalamus, amygdala, cingulate cortex, and insula[13].

One study found that activity in the cingulate cortex and insula correlated with subjective arousal in women observing an erotic film[17], however no genital arousal metrics were recorded and it has been demonstrated that subjective and genital arousal are highly desynchronous in women[9]. Similarly, studies of the thalamus have only evaluated its activation correlated to female subjective arousal or male erection[17,18], but not genital arousal. The thalamus receives sensory information from the genitals via the spinothalamic tract[19]. As such, the thalamus has been regarded as a relay center for desire, arousal, and orgasm[13].

The hypothalamus is the only identified supraspinal region with research that directly confirm its involvement in female genital arousal. The medial preoptic area (MPOA) of the hypothalamus is known to be involved in sexual arousal and desire[20] and is the target area for the hypoactive sexual desire disorder (HSDD) drug bremelanotide. At least one study has shown that stimulation of the MPOA leads to an increase in vaginal blood flow[21]. From the MPOA, there are projections to the periaqueductal gray (PAG) of the midbrain. The PAG also receives input from additional cortical structures related to sexual function (such as other hypothalamic nuclei and the amygdala) as well as afferents from the pelvic organ relay center (PORC) via the

spinothalamic tract. The PAG projects efferents to the pelvic organ stimulating center (POSC) and pelvic floor stimulating center (PFSC), both of which are located in the pons[22]. The role of the POSC in genital arousal is currently unclear and has been studied mostly with respect to micturition. Because descending projections to sacral parasympathetic motoneurons (which gives rise to the pelvic nerve) have only been found in the POSC, it is likely that this pathway is heavily involved in controlling genital arousal[22,23], however this has not yet been confirmed explicitly.

More is understood about the brain regions that are involved in orgasm than genital arousal. An fMRI study showed that several of the brain regions putatively involved in arousal are also activated during orgasm: the hypothalamus, amygdala, cingulate cortex, and insula[24]. Although not explicitly studied with respect to genital arousal, the amygdala encodes emotional significance to events, including erotic stimuli, and has inhibitory GABAergic projections to the hypothalamus[13,25,26]. Thus, it is likely it has some influence over genital arousal via the hypothalamic pathways discussed above. Both pelvic stimulating centers are activated during orgasm[27], but only the PFSC has efferents to somatic motoneurons that innervate the pelvic floor muscles. Supporting its role as a descending pathways, stimulation of the PFSC led to contractions of the pelvic floor muscles[28].

There are a wide variety of neurotransmitters involved with hypothalamic activity, including serotonin, dopamine, acetylcholine, norepinephrine, glutamate, and GABA. While the specifics of how they relate to genital arousal are unclear or only studied in males, broad generalizations have been found for women. Serotonin decreases arousal and facilitates orgasm, while dopamine has the opposite effect[10]. Norepinephrine increases both arousal and orgasm,

while the effects of acetylcholine are inconclusive[10]. Nitric oxide plays an important role in vasodilation during genital arousal[29].

The autonomic nervous system is responsible for the direct control of the genitals, apart from the striated pelvic floor muscles which are under voluntary control via the pudendal nerve. The female sexual response cycle has both sympathetic and parasympathetic autonomic influence, however the way these two systems interact is not yet fully understood. Parasympathetic activation during sexual activity causes relaxation of the smooth muscles in the muscularis of the vaginal wall and the corpus cavernosum of the clitoris which causes vasodilation, increasing blood flow to the genitals. Increased blood flow leads to the production of transudate in the vaginal wall which contributes to lubrication that is necessary for painless penetrative intercourse. This activation is mediated by the pelvic nerve whose preganglionic fibers arise from the S2-S4 levels of the spinal cord. The pelvic nerve's preganglionic cell bodies project from the POSC via the intermediolateral column[30]. The parasympathetic fibers then synapse in the pelvic plexus, also referred to as the inferior hypogastric plexus (Figure 1) which is located on the surface of the pelvic viscera. Postganglionic parasympathetic fibers go on to innervate the vagina and clitoris. Both pre- and postganglionic fibers of the pelvic nerve, as with all parasympathetic nerves, are cholinergic. The pelvic plexus also receives sympathetic input from the hypogastric

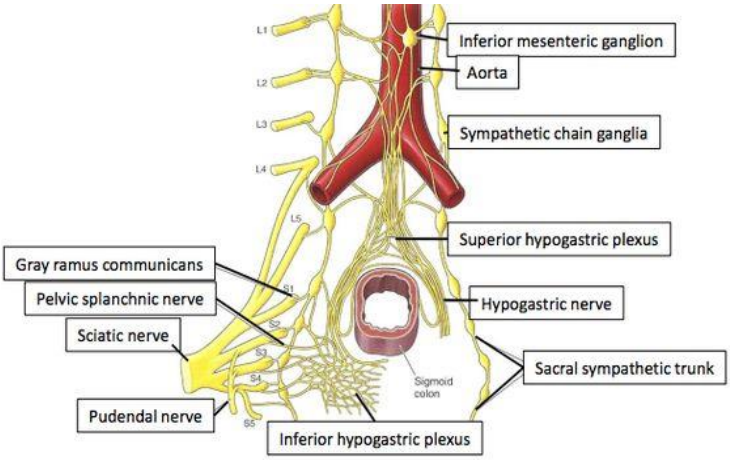

Figure 1. An overview of the neuroanatomy of the autonomic and somatic nerves involves in genital arousal and orgasm. The sympathetic chain ganglia, superior hypogastric plexus, and hypogastric nerve are part of the sympathetic nervous system, and the pelvic (splanchnic) nerve is part of the parasympathetic nervous system. The inferior hypogastric plexus receives both parasympathetic and sympathetic efferents and the pudendal nerve is part of the somatic nervous system.

nerve and sympathetic chain (see Figure 1), however these sympathetic efferents do not synapse in the plexus like the pelvic nerve. The hypogastric nerve arises from the superior hypogastric plexus, whose preganglionic fibers arise from the T11-L2 levels of the spinal cord. Similar to the pelvic nerve, the hypogastric has preganglionic cell bodies located just medial of the intermediolateral column and its sensory afferents project as the posterolateral tract in felines[31,32]. However, a significant difference is that the postganglionic fibers of the sympathetic nervous system are adrenergic. This explains findings from an organ bath study that dosing with norepinephrine led to contraction of vaginal and clitoral tissue as well as a human study that saw administration of adrenergic agonists suppresses vaginal blood flow and lubrication[14]. These sympathetic efferents are responsible for innervation of the blood vessels and their excitation causes vasoconstriction, which leads to decreased vaginal blood flow.

The pudendal nerve provides the only voluntary control of the pelvic organs and arises from the nucleus of Onuf. This has been shown via retrograde tracing and functional studies that stimulated the nucleus of Onuf which caused an increase in pelvic floor muscle activity (same effect as stimulation of the pudendal nerve and PFSC)[28,33]. The pudendal nerve exits the spinal cord at the S2-S4 levels and goes on to innervate the striated pelvic floor muscles, as well as the external urethral and anal sphincter. The pudendal nerve most notably has a role in orgasm, since it involved a rhythmic contraction of the pelvic floor, but stimulation of its sensory fibers lead to an increase in vaginal blood flow[34]. This blood flow response is abolished after bilateral pelvic nerve transection, suggesting that there are spinal pathways driven by pudendal afferent activity that mediate parasympathetic control of genital arousal.

Although norepinephrine and acetylcholine are considered the classic neurotransmitters of the autonomic nervous system, several other neurotransmitters are released by the nerves

innervating the pelvic organs and may have more influence over genital blood flow[35]. Vasoactive intestinal polypeptide (VIP) promotes vasodilation which increases muscle relaxation and when administered in humans, increases vaginal blood flow[36]. Nitric oxide (NO) also promotes vasodilation and acts on receptors of the smooth muscle in the vagina and clitoris[37]. The release of NO and VIP seems to occur in the cholinergic neurons, consistent with the facilitation of vaginal blood flow by the parasympathetic nervous system[38].

The vagus nerve hypothesized as having a role in sexual function, however the details are sparse. The theory first arose when a study demonstrated that certain physiological responses to vaginocervical stimulation remained after bilateral transections of the pelvic, hypogastric, and pudendal nerve in rats. These responses were only abolished after bilateral vagotomy[39]. This is evidence of a pathway that circumvents the lumbosacral spinal cord and may explain how women with complete spinal cord injury are able to experience genital sensations[40]. Later fMRI studies in humans showed that the nucleus of the solitary tract, a region to which the vagus nerve projects, is activated during orgasm[24]. This confirms the retrograde tracing of the cervix using horseradish peroxidase which labeled the nodose ganglion[41].

1.1.3 Diagnosing and treating female sexual dysfunction

Female sexual dysfunction is an umbrella term that can refer to a medical diagnosis or a poor score on a validated sexual function survey. Medical diagnoses include disorders and dysfunctions such as vulvodynia, genitourinary symptoms of menopause, or female sexual interest and arousal disorder among many others. There are several validated surveys for evaluating female sexual function, such as the female sexual distress scale (FSDS)[42] and the female sexual function index (FSFI)[43]. The FSFI has scores that range from 0 to 36 (no dysfunction) and asks about sexual function in six sub-domains: desire, arousal, lubrication, pain,

orgasm, and satisfaction. Roughly 40% of women report having sexual dysfunction when evaluated by the FSFI[44], which has a clinical cut-off score of 26.55[45]. Unfortunately, there are few treatment options for FSD, despite its strong ties with overall life satisfaction[1]. This is due, in part, to the lack of comprehensive, quantitative metrics to assess sexual health and further differentiate the various etiologies of FSD.

One of the first and most established quantitative measures of female sexual arousal is vaginal photoplethysmography (VPG). VPG measures genital arousal as the peak-to-peak amplitude of the blood flow signal associated with each heartbeat measured in the vaginal canal. The VPG device contains a light-emitting diode and photosensitive light detector that measures the backscatter and assumes a direct correlation with vaginal vasocongestion. VPG is limited in its use because it is subject to movement artifacts and low sexual concordance, something we also observed in our experiments in Chapter 3 and 4. A relatively new alternative to VPG is laser speckle contrast imaging (LSCI) which produces a heatmap of the vaginal vestibule that correlates to superficial blood flow. LSCI and other external laser-based devices for assessing genital arousal are gaining favor over traditional plethysmography because they measure vulvar and clitoral blood flow and the device is not in contact with the body, reducing movement artifacts and allowing for more comfort during a sensitive measurement. There is also preliminary evidence that vulvar blood flow has a higher agreement with subjective arousal than vaginal blood flow, which may indicate a more comprehensive assessment of female sexual function[46].

Despite the prevalence of FSD, existing treatment options lack a targeted approach or are marginally effective. Traditional talk therapy is commonly used to treat psychological dysfunctions such as low libido or hypoactive sexual desire disorder (HSDD) but cannot address

impaired sexual arousal if there is underlying pathophysiology. Flibanserin and bremelanotide are the only pharmaceuticals approved to treat HSDD, approved in 2015 and 2019 respectively, and have not yet gained wide-scale adoption. Flibanserin is a daily pill and has shown to be mildly effective at treating HSDD in post-menopausal women, but there has been controversy due to its high incidence of adverse events[47] and that it only improves the desire sub-domain of the FSFI[48]. Bremelanotide is a subcutaneous injection given at least 45 minutes before sexual activity and is limited to 8 doses per month. Clearly there are some limitations related to its administration and it also has a high incidence of adverse events, the most common one being nausea[49]. Neither drug has shown evidence for improving genital arousal.

Sildenafil, a popular treatment for erectile dysfunction under the trade name Viagra, is sometimes prescribed off-label to improve sexual arousal in people with vulvas. Studies have found that sildenafil can increase vaginal and clitoral vasocongestion[50,51], but the clinical relevance is unknown because genital vasocongestion did not always translate to increase subjective arousal or satisfying sexual experiences[52]. Finally, hormone therapy is common but generally reserved for post-menopausal women[53], leaving out as much as 20% of pre-menopausal women who have a sexual disorder[54]. Neuromodulation, or targeted electrical stimulation, is emerging as a potential treatment for FSD and is the focus of this dissertation. We will discuss this in-depth in section 1.2, but first, discuss how spinal cord injury (SCI) impairs sexual function in people who are assigned female at birth.

1.1.4 Spinal cord injury and female sexual dysfunction

Women with spinal cord injury (SCI) account for roughly 20% of the 300,000 Americans living with SCI[55]. An estimated 42.5% of all SCI patients are classified by the American Spinal Injury Association Impairment Scale (AIS) as AIS-A (complete) and 52.4% classified as AIS-B

through AIS-D (incomplete)[56]. Classification of SCI is determined by how much sensory and motor function remains and spans from AIS-A (no sensory or motor function present) to AIS-D (intact sensory and functional motor function) to AIS-E (normal function)[57]. Among paraplegics, regaining sexual function is one of the higher priorities, and for quadriplegics, it falls only second to regaining arm and hand function[2,3]. Despite this, women with SCI have the same, limited treatment options as women with non-neurogenic FSD.

Studies have shown that women with SCI have similar levels of desire as non-neurogenic women, but the impact of SCI on genital blood flow, arousal, and orgasm is not fully understood[58]. Vaginal vasocongestion, subjective arousal, and the ability to orgasm after injury varies greatly depending on injury severity and location. For example, the ability to achieve orgasm can remain in women with complete SCI[58]. Although the time to achieve orgasm takes significantly longer, the qualitative characteristics of the orgasm itself remain indistinguishable from those in non-neurogenic counterparts. One hypothesis for how orgasm is maintained is that sacral arc reflex (i.e. orgasm reflex) is controlled in part by descending inhibitory control that is abolished after spinal cord transection[59]. Another hypothesis is that orgasm is mediated in part by vagal afferents relayed through the pelvic plexus, also referred to as the pelvic ganglion in animals[24,40,60,61]. Finally, it is also possible that there are some spinal pathways left intact, even in the case of AIS-A patients[62]. The autonomic signals controlling sexual function descend from the POSC through the reticulospinal tract, which may remain intact depending on the location and severity of SCI. Recall that complete SCI or AIS-A is characterized by a lack of sensation and motor control, which does not necessarily mean that all autonomic function is destroyed. Thus, neuromodulation may be a viable treatment by which to rehabilitate the injured neural pathways. This is supported by research that shows that the more T11-L2 dermatomes that remain intact,

the more likely women with SCI will be able to achieve psychogenic arousal[58]. If the circuitry by which neuromodulation improves FSD in women without SCI remains intact, it is a potential treatment option for women with incomplete SCI as well.

1.2 Neuromodulation

Sacral neuromodulation (SNM) has been approved to treat bladder dysfunction for decades[63]. SNM involves surgically placing an implantable pulse generator and stimulation leads near the sacral roots, located near the tailbone. Stimulation is delivered continuously and modulates bladder function by activating different neural pathways via the sacral nerves. The exact mechanisms are unknown, but we know it involves activation a sacral reflex arc, afferent pathways, and even some CNS pathways[64]. SNM is a relatively expensive and invasive surgical procedure and a temporary solution, as the implanted pulse generator has a limited life-span and requires replacement surgeries[65]. Other neural targets such as the genital and tibial nerve are studied to treat lower urinary tract (LUT) dysfunctions via percutaneous and transcutaneous stimulation[1,2]. A review of SNM found significant improvements in female sexual function, so it stands to reason genital nerve stimulation (GNS) and tibial nerve stimulation (TNS), as distal branches of sacral roots, are worth exploring to treat FSD as well. A review of percutaneous tibial nerve stimulation (PTNS) supports this and found significant increases in survey reported sexual function[66].

1.2.1 Genital and tibial neuromodulation

Currently, most uses of GNS are for bladder and bowel dysfunction. One pilot investigation found that repeated transcutaneous GNS led to increases in the arousal, lubrication, and orgasm domains of the FSFI[67]. The genital nerve is a terminal branch of the pudendal nerve

and innervates the clitoris. Rodent studies have shown sensory pudendal stimulation leads to vaginal vasocongestion[34,68]. Perhaps GNS evokes a similar response as pudendal nerve stimulation and improved survey scores are due to improvements in genital blood flow.

The tibial nerve's role in sexual function is unclear, but TNS is routinely used to treat overactive bladder[65]. It is hypothesized that TNS inhibits bladder contractions and increases bladder volume via CNS modulated sacral afferents and lower urinary tract efferents[69]. It is possible that TNS could modulate sexual function given the shared central and peripheral neuroanatomy of the pelvic organs. Preliminary studies have shown TNS can improve survey-reported female sexual function[70]. However, only one study has demonstrated this in women without concomitant pelvic organ dysfunction and it did not quantify genital arousal[67]. However, TNS studies in rodents have shown that TNS can drive genital arousal[71]. This suggests that TNS may also improve sexual function by increasing genital vasocongestion, but further studies to understand the mechanisms are needed.

1.2.2 Spinal cord injury and neuromodulation

On average, participants with SCI reported bladder and bowel function as a high priority to regain, with similar levels as sexual function[2,3]. Neither GNS nor TNS has been studied in people with SCI to treat sexual dysfunction, but we can infer its potential from studies on urinary and fecal incontinence[72]. When used as an early intervention, TNS has been shown to be effective in preventing urinary incontinence and improving bladder capacity in patients with SCI[73,74]. Acute GNS has shown to be effective in suppressing bladder overactivity in patients over a year post-injury, likely through different mechanisms[75]. Another retrospective study evaluating the effect of sacral neuromodulation on pelvic floor dysfunctions after SCI showed an improvement in erectile dysfunction[76]. These examples of GNS, TNS, and SNM to treat bladder

and male erectile dysfunctions, and others[72], support neuromodulations potential to treat FSD after SCI.

1.3 Dissertation Work

Neuromodulation is often successful in treating LUT dysfunction, but the exact mechanisms are not well understood. It stands to reason that the same neuromodulation therapies that are effective at treating bladder dysfunction may help FSD because of the pelvic organs' shared neuroanatomy and preliminary studies on sexual function. Clinical studies have survey-reported outcomes on the repeated effect of transcutaneous genital and tibial nerve stimulation on sexual function but lack quantitative metrics on genital arousal. Animal studies support genital arousal can be modulated by pudendal and tibial nerve stimulation, but only vaginal blood flow has been researched despite the vulva and clitoris playing important roles in female sexual function.

My dissertation research combines genital arousal metrics and existing knowledge of LUT neuromodulation in a novel way to demonstrate the potential of TNS and GNS to treat FSD. In Chapter 2, I show that pudendal, but not tibial nerve stimulation, can modulate vulvar blood perfusion in an anesthetized rodent model, indicating the need for anatomical specificity in our genital arousal metrics. In Chapter 3, I demonstrate how women with complete SCI can experience genital sensations in response to acute TNS and GNS and that traditional clinical measurement techniques yield low sexual concordance in the absence of erotic stimuli, consistent with previous research findings[9]. In Chapter 4, I found that GNS and TNS can increase the VPA response to erotic stimuli and that sexual concordance varies across participant groups.

Genital arousal and subjective arousal responses to stimulation varied within and across studies. We found that participants with complete SCI could experience genital sensations in response to GNS and TNS. Most participants indicated willingness to use transcutaneous electrical stimulation. This dissertation provides further support for GNS and TNS as a treatment for female sexual dysfunction.

Chapter 2 Pudendal, but Not Tibial, Nerve Stimulation Modulates Vulvar Blood Perfusion in Anesthetized Rodents

(Previously published in the International Urogynecology Journal, September 2022[77])

2.1 Abstract

Introduction and Hypothesis: Preclinical studies have shown that neuromodulation can increase vaginal blood perfusion, but the effect on vulvar blood perfusion is unknown. We hypothesized that pudendal and tibial nerve stimulation could evoke an increase in vulvar blood perfusion.

Methods: We used female Sprague-Dawley rats for non-survival procedures under urethane anesthesia. We measured perineal blood perfusion in response to twenty-minute periods of pudendal and tibial nerve stimulation using laser speckle contrast imaging (LSCI). After a thoracic-level spinalization and a rest period, we repeated each stimulation trial. We calculated average blood perfusion before, during, and after stimulation for three perineal regions (vulva, anus, and inner thigh), for each nerve target and spinal cord condition.

Results: We observed a significant increase in vulvar, anal, and inner thigh blood perfusion during pudendal nerve stimulation in spinally intact and spinalized rats. Tibial nerve stimulation had no effect on perineal blood perfusion for both spinally intact and spinalized rats.

Conclusions: This is the first study to examine vulvar hemodynamics with LSCI in response to nerve stimulation. This study demonstrates that pudendal nerve stimulation modulates vulvar blood perfusion, indicating the potential of pudendal neuromodulation to improve genital blood flow as a treatment for women with sexual dysfunction. This study provides further support for neuromodulation as a treatment for women with sexual arousal disorders. Studies in

unanesthetized animal models with genital arousal disorders are needed to obtain further insights into the mechanisms of neural control over genital hemodynamics.

2.2 Introduction

Female sexual health is an important determinant in quality of life, contributing to both increased meaning in life and general well-being[78]. Unfortunately, approximately 40% of women suffer from female sexual dysfunction (FSD)[44]. Female sexual dysfunction can present in multiple domains, which can be thought of as physiological (arousal, lubrication, orgasm, pain) and psychological (satisfaction and desire). Due to limited research regarding the etiology of FSD and basic female anatomy, current treatment options for FSD are very limited, especially for women who have deficits in the physiological domains such as arousal[9]. Bremelanotide and flibanserin, both approved to treat hypoactive sexual desire disorder (HSDD), have shown mild efficacy in improving sexual desire and arousal[79,80]. However, bremelanotide has been associated with a high incidence of adverse events[81] and a review of flibanserin, suggests that it has minimal clinical benefit[48]. Sildenafil citrate has been studied as a treatment for female arousal disorders. One study of sildenafil demonstrated an increase in clitoral vasocongestion and an association with increased sexual satisfaction[51]. Sildenafil was also used to study vaginal vasocongestion in treatment and placebo groups during erotic visual stimulation[82]. Although this study reported significant increases in vasocongestion for the treatment group, there were no differences in subjective arousal between the sildenafil and placebo groups. The lack of consistent improvements in subjective arousal as well as a high incidence of adverse events led to sildenafil citrate no longer being pursued as a treatment option[83].

Neuromodulation is a potential treatment option for women with FSD. Sacral neuromodulation (SNM) is a standard treatment for overactive bladder and fecal

incontinence[84,85]. SNM delivers electrical stimulation to sacral nerves, which contain somatic and sympathetic nerve fibers of the pelvic organs. Clinical studies have found that sexual function can improve in women receiving SNM for bladder function[86,87]. Similar improvements in sexual function have been seen in a clinical study that used tibial nerve stimulation to treat bladder dysfunction[88]. The sexual health benefits of neuromodulation have been studied in women without concomitant pelvic disorders. In a recent study, transcutaneous electrical nerve stimulation (TENS) of the tibial nerve or genital nerve, a distal branch of the pudendal nerve, improved survey-reported female sexual function index (FSFI) scores, a standardized metric for evaluating female sexual function [67]. These subjects reported an improvement in their overall FSFI score and their individual arousal and orgasm sub-scores. Subjects receiving DGN stimulation also saw improvements in sexual satisfaction. There is a lack of physiological measurements of sexual health and so the mechanisms by which pudendal and tibial nerve stimulation improve sexual function are not completely understood. One possible mechanism is that nerve stimulation modulates genital blood flow, which is necessary to facilitate vasocongestion, lubrication, and sexual receptivity[89]. This is supported by preclinical studies showing that stimulation of the tibial nerve for 30 minutes can result in a transient increase in vaginal blood flow 20-35 minutes after stimulation onset[71]. Preclinical studies have also shown an increase in vaginal blood flow in response to pudendal nerve stimulation, with peak responses occurring anywhere from 1-2 minutes[34] or up to 30 minutes[68] after stimulation onset depending on stimulation parameters. Additionally, it is well established that stimulation of the somatic pudendal nerve and tibial nerve can modulate spinal control over the bladder and bowel, leading to improvement in dysfunctional states[90]. We hypothesized that a similar spinal reflex exists for genital vasocongestion. Both pudendal and tibial nerve stimulation have the potential to improve

sexual function and have specific translational advantages, including the ease of access and existing clinical use for pelvic dysfunctions[88,91], however a greater understanding of the underlying mechanisms and neural circuitry is needed.

Female sexual dysfunction often presents in women with spinal cord injuries (SCI). Women with SCI retain varying aspects of sexual function, depending on their injury type. For example, research has shown that sensory impairment to the T11-L2 dermatomes, but not injury level, is associated with decreased genital arousal[58]. Although paraplegic patients report that regaining sexual function after SCI is a priority[92], women with SCI have the same FSD treatment options as non-neurogenic women. These treatment options do not consider the impact of neurological dysfunction. Furthermore, neuromodulation via tibial nerve stimulation may need supraspinal pathways[90] which would limit utility for SCI women depending on their injury severity. Once the neural pathways controlling genital arousal are better understood, clinicians can use knowledge of the patient's injury to determine if electrical stimulation is able modulate these pathways to improve genital arousal in women with neurogenic FSD. However, it is first necessary to identify how different nerve targets of neuromodulation modulate genital arousal. In healthy women, genital arousal is a physiological response characterized by an increase in genital blood flow, engorgement of the genitals, and the production of lubrication[89]. Most research studying female sexual responses use vaginal photoplethysmography to measure genital arousal[93]. However, some researchers are turning towards more non-invasive methods such as laser doppler imaging or laser speckle contrast imaging (LSCI). These laser-based methodologies are becoming more useful in measuring female sexual arousal because they show a higher concordance between genital and subjective arousal[94]. However, the importance of genital-subjective arousal synchrony is not well understood[9] and some models for the sexual response

cycle suggest that genital arousal is necessary for satisfactory sexual intercourse, regardless of subjective arousal[95].

In this preclinical study we used LSCI to measure blood perfusion changes in the perineal region of female rats in response to tibial and pudendal nerve stimulation. Additionally, we looked at how this blood perfusion response changes after thoracic level spinalization. Although LSCI has been used to assess genital arousal in clinical studies[94,96], this is the first time it has been used to measure perineal blood perfusion in animals. Our main outcome measures for this study were changes in vulvar, anal, and inner thigh blood perfusion from baseline in response to nerve stimulation. We hypothesized that pudendal nerve stimulation would drive larger increases in vulvar blood perfusion than tibial nerve stimulation. We also hypothesized that spinal cord transection would decrease the perineal blood perfusion response to tibial nerve stimulation, but not pudendal nerve stimulation.

2.3 Methods

2.3.1 Animal Surgery

All experimental procedures were approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC) in accordance with the National Institutes of Health's guidelines for the care and use of laboratory animals. We designed the primary experimental protocol prior to starting the study. The study protocol was not pre-registered. We performed non-survival procedures on 17 nulliparous female Sprague-Dawley rats (Charles River Breeding Labs, Wilmington, MA, USA) weighing 0.25 to 0.30 kilograms. Female Sprague-Dawley rats are a common preclinical model for studying sexual arousal because their physiological response to sexual stimuli is similar to that found in humans (e.g., increases in vaginal blood flow, length,

and pressure)[97]. Additionally, the neuroanatomical pathways of the pelvic, hypogastric, and pudendal nerves, all which mediate genital arousal and sensation, have been well studied in rats[98]. We performed a power analysis using a Wilcoxon signed-rank test with $\alpha = 0.05$, power = 0.80, and an effect size estimate from genital perfusion changes in a study with women[99] that resulted in a sample size of 9. We targeted 15 rats to account for the potential for higher variability in animal genital perfusion responses and possible animal deaths under anesthesia. Ultimately we had 10 animals that completed the full set of experiments, which matches prior animal studies with similar objectives[68,100]. Animals were housed in ventilated cages under controlled temperature, humidity, and photoperiod (12-h light/dark cycle), and provided laboratory chow (5L0D, LabDiet, St. Louis, MO, USA). Animals were anesthetized using intraperitoneal urethane (1.5 g/kg), a commonly used anesthetic in rodent surgeries studying pelvic organ function[34,101]. Sufficient anesthetic depth was confirmed once the animal no longer had a toe pinch response. We used a heating pad to maintain body temperature and monitored vital signs (heart rate, respiration rate, and oxygen saturation levels) every 15 minutes. We used vital signs as humane endpoints. We performed vaginal cytology prior to surgical access to determine estrous stage, to examine if one stage (e.g. estrus) better facilitates a perineal blood flow response.

With the rat in the prone position, we made a dorsal midline incision through the skin 3-4 cm rostral to the base of the tail. Then we extended the incision laterally on the right side, and the ischiorectal fossa was separated. We used retractors to keep the musculature open and isolated the pudendal nerve using forceps. We placed a bipolar nerve cuff with stranded stainless-steel wire electrodes (400 μm diameter; Cooner Wire Co, Chatsworth, CA, SA) and silicone elastomer tubing (0.5-mm inner diameter; Dow Corning, Midland, MI, USA) around

both sensory and motor branches of the pudendal nerve within the ischiorectal fossa, proximal to the division of the motor branch into its dorsal and ventral branches[102]. We then closed the incision and moved the animal to a supine position. We then placed a percutaneous electromyogram (EMG) wire (stainless steel, 50 μm, MicroProbes, Gaithersburg, MD) subcutaneously, parallel to the tibial nerve and ipsilateral to the pudendal nerve cuff. We measured perineal blood perfusion with a laser speckle contrast imaging (LSCI) system (MOORFLPI-2, Moor Instruments, Wilmington, DE). We aimed the laser at the perineal region, angled downward at 25 degrees, and positioned such that the path of the laser traveled 15 to 20 cm to the animal (Figure 2A).

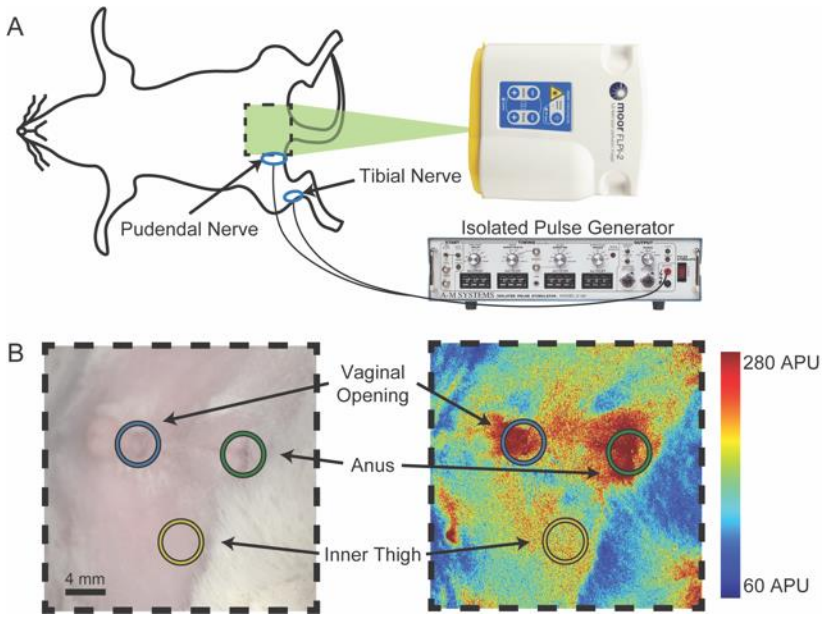

Figure 2. Experimental set-up. A) A diagram of the LSCI with the laser aimed at the vaginal vestibule of a rat lying in the supine position. B) An example of a laser speckle imaging live (*left*) and produced as a heatmap (*right*) with ROIs drawn around the vaginal vestibule, anus, and a region in the inner thigh in *blue*, *green*, and *yellow* respectively.

2.3.2 Experimental Protocol

We delivered stimulation using an isolated pulse generator (Model 2100, A-M Systems, Sequim, WA). We determined the motor threshold (MT) for stimulating the pudendal and tibial nerves by slowly increasing the stimulation amplitude until a motor response (anal or toe twitch respectively) was observed. Each animal went through a series of 45-minute trials. For each trial, we used LSCI to measure perineal blood perfusion at the maximum sampling rate of 0.25 Hz, using the temporal processing settings of the MOORFLPI-2, for 5 minutes prior to stimulation,

during 20 minutes of stimulation, and for 20 minutes after stimulation. We delivered stimulation to one of the nerve targets using biphasic, rectangular pulses (0.2-ms pulse width) at 20 Hz and twice the MT. We repeated the recording protocol for the other nerve target. After both trials, we placed the rat in the prone position and performed a laminectomy. We used a rostral/caudal incision to expose T8-10 level vertebrae. After removal of the dorsal processes, we used a scalpel blade to transect the spinal cord. We closed the musculature with sutures and closed the skin with skin staples. We then moved the rat to the supine position and allowed it to rest for at least 15 minutes. Next, we re-assessed the MT and repeated the trials in the same order as the intact spinal cord trials. After all experimental procedures were complete, we euthanized the animal with an intraperitoneal injection of sodium pentobarbital (250-300 mg/kg). In the first three rats, tibial nerve stimulation was performed first. We alternated the order of the first stimulated nerve across subsequent animals, occasionally using sequential animals with pudendal nerve stimulation first, to arrive at balanced groups upon study termination. Investigators were not blind to stimulation order.

We added two sets of supplemental experiments to the protocol after the study began. In some initial experiments, we saw the blood perfusion signal spontaneously increase over 100 APU for 1 to 3 minutes, at 5-to-6-minute intervals. These increases were too abrupt to be considered a physiological response in blood perfusion, so we performed another set of experiments to examine LSCI data sampling (n = 2). The experimental procedure in these experiments followed the same set of 4 trials (pudendal and tibial stimulation, before and after spinal cord transection), except that the blood perfusion was sampled at 1 Hz or 25 Hz using the spatial processing settings instead of the maximum sampling rate of 0.25 Hz with the temporal processing setting. In the second set of supplemental experiments (n = 3), we transected the

pudendal nerve next to the cuff electrode between stimulation trials with an intact spinal cord. We looped a suture string around the nerve during cuff placement and pulled it tightly to cut the nerve. We transected the pudendal nerve a different way in each of these experiments: once proximal to the cuff, once distal to the cuff, and once distal to the cuff with a partial and then complete distal cut.

2.3.3 Data Analysis

We performed all data and statistical analyses using moorFLPI-2 Research Software (Software-MOORFLPI2-3VX, Moor Instruments, Wilmington, DE) and MATLAB (Mathworks, Natick, MA, USA). We drew regions of interest (ROIs) around the vulva, the anus, and a region on the inner thigh for each LSCI trial analysis in moorFLPI-2. We drew a circular ROI centered on the vaginal orifice, with a diameter equal to the width of the tissue mound surrounding the urethra. We then centered two identical sized ROIs on the anus and the inner thigh on the contralateral side of where stimulation was delivered, such that the three ROIs formed an equilateral triangle (Figure 2B). We calculated average perfusion units within each ROI (vulvar, anal, and inner thigh) and extracted the signal for each 45-minute trial. Each signal is referred to as vulvar blood perfusion (VBP), anal blood perfusion (ABP), and inner thigh blood perfusion (ITBP). We observed an artifact when stimulation was turned on and removed it by eliminating the data from 20 seconds before to 60 seconds after stimulation onset. We then calculated the temporal mean for each ROI before, during, and after stimulation. We used a linear regression to determine the impact of estrous stage, stimulation order, experiment number, and weight on the results. Estrous stage and stimulation order were coded as binary values in this analysis. We made comparisons between average VBP, ABP, and ITBP before and after spinalization as well

as across stimulation epochs (before, during, and after the stimulation periods) using t-tests (alpha = 0.05). Each animal served as their own control.

2.4 Results

We considered animals that completed all 4 nerve stimulation trials (n = 10) as experimental units and used them for full data analysis. Three animals were used to perform supplementary nerve transection trials. Two animals had blood perfusion measured using different LSCI parameters to test for aliasing. The remaining two animals died prematurely during the second stimulation trial and were excluded from analysis. Estrous stage, stimulation order, experiment number, and weight had no impact on results. Detailed experimental demographics can be found in Table 1. Experimental data and MATLAB scripts used in the data analysis are available online[103].

Table 1. Summary of experiment parameters

				Intact Spinal Cord		Spinalized	
Animal ID	Weight (kg)	First Nerve Target	Estrous Stage	Pudendal Nerve MT (μA)	Tibial Nerve MT (μA)	Pudendal Nerve MT (μA)	Tibial Nerve MT (μA)
A	0.29	Tibial	Diestrus	300	400	500	1000
B	0.29	Tibial	Inconclusive	3000	3600	2100	1800
C	0.29	Tibial	Proestrus	500	300	500	300
F	0.29	Pudendal+	Metestrus	500	1300	500	3400
G	0.30	Tibial+	Metestrus	400	250	400	900
J	0.28	Pudendal	Proestrus	300	600	500	5000
K	0.26	Pudendal	Proestrus	200	400	1000	400
M	0.25	Tibial	Inconclusive	300	750	600	600
N	0.26	Pudendal	Proestrus	130	400	300	400
O	0.26	Pudendal	Inconclusive	750	1700	750	1500
P	0.26	Tibial	Estrus	140	250	170	700
Q	0.26	Pudendal*	Estrus	170	-	-	-
T	0.25	Tibial	Diestrus	260	110	400	530
U	0.26	Pudendal*	Metestrus	110	-	-	-
V	0.26	Pudendal*	Inconclusive	600	-	-	-

MT: motor threshold, *: Nerve Transection Experiment, +: Sampling Frequency Experiment

2.4.1 Intact Spinal Cord Recordings

During pudendal nerve stimulation trials (Figure 3a), the average VBP at baseline was 184 ± 44 APU, which increased to 331 ± 129 APU during stimulation (+80.9 ± 64.2% change from baseline) and fell to 185 ± 55 APU after stimulation. Similarly, the average ABP was 172 ± 40 prior to stimulation, 348 ± 164 during stimulation (+97.6 ± 74.4%), and 169 ± 44 APU after stimulation ended. The average ITBP was 94 ± 33, 154 ± 67 (+67.0 ± 63.7%), and 92 ± 35 before, during, and after pudendal stimulation. The average blood perfusion during pudendal nerve stimulation for all ROIs was significantly higher than at baseline and after stimulation ($p < 0.01$).

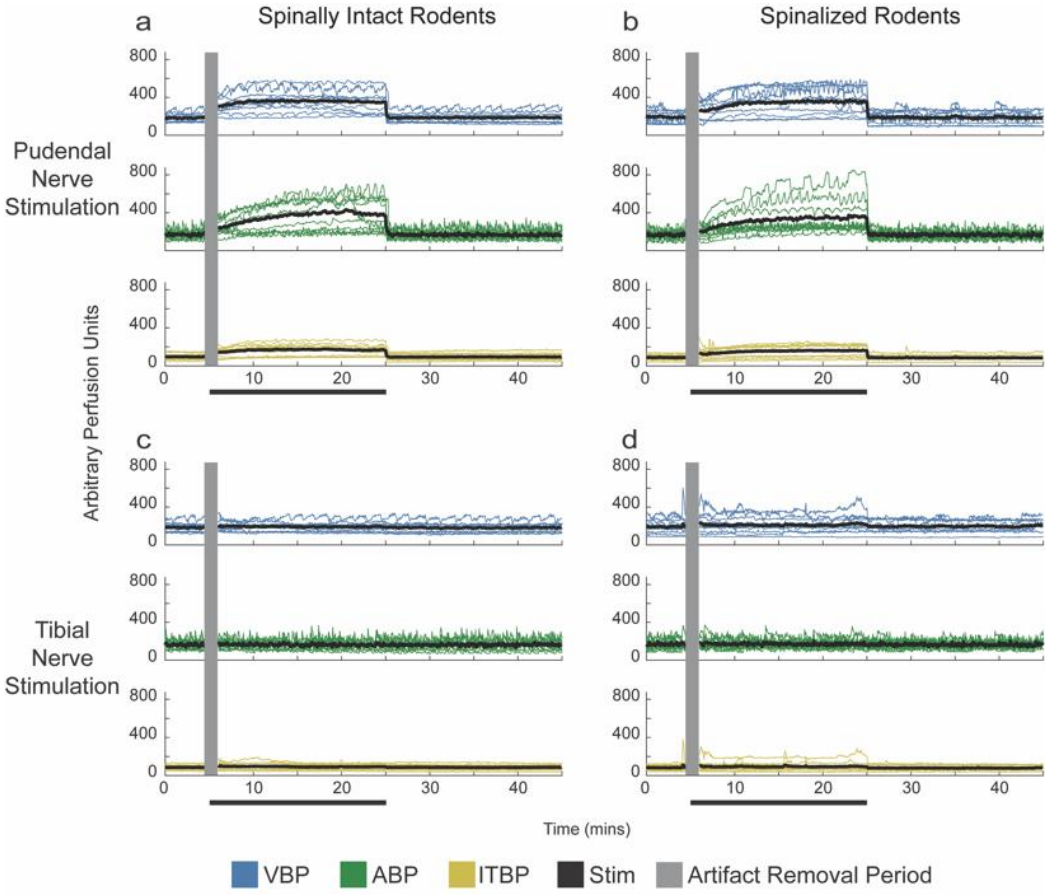

Figure 3. Individual (thin, colored lines) and average (solid black line) blood perfusion responses for all animals that completed the entire nerve stimulation protocol (n = 10). a) Pre-spinalization pudendal stimulation trials. Blood perfusion returned to baseline after stimulation was turned off at 25 minutes. b) Pudendal stimulation trials after spinalization follow similar trends as pre-spinalization trials. Tibial nerve stimulation trials for intact (c) and spinalized (d) trials.

The average VBP for tibial nerve stimulation trials (Figure 3c) was 183 ± 50 APU prior to stimulation, 192 ± 48 APU during stimulation (+5.7 ± 11.9%), and 181 ± 52 APU after stimulation ended (Figure 4c). For the same trials, ABP was 163 ± 42, 163 ± 42 (+0.4 ± 8.0%), and 162 ± 47 APU before, during, and after stimulation. ITBP had mean values for the three periods of 85 ± 26, 93 ± 34 (+7.8 ± 22.5%), and 85 ± 31 APU. Tibial nerve stimulation did not elicit significant changes in any ROIs during stimulation, and the average blood perfusion was significantly lower in all ROIs compared to pudendal nerve stimulation ($p < 0.001$). Neither pudendal nor tibial stimulation elicited changes in perineal blood perfusion that persisted after stimulation was turned off.

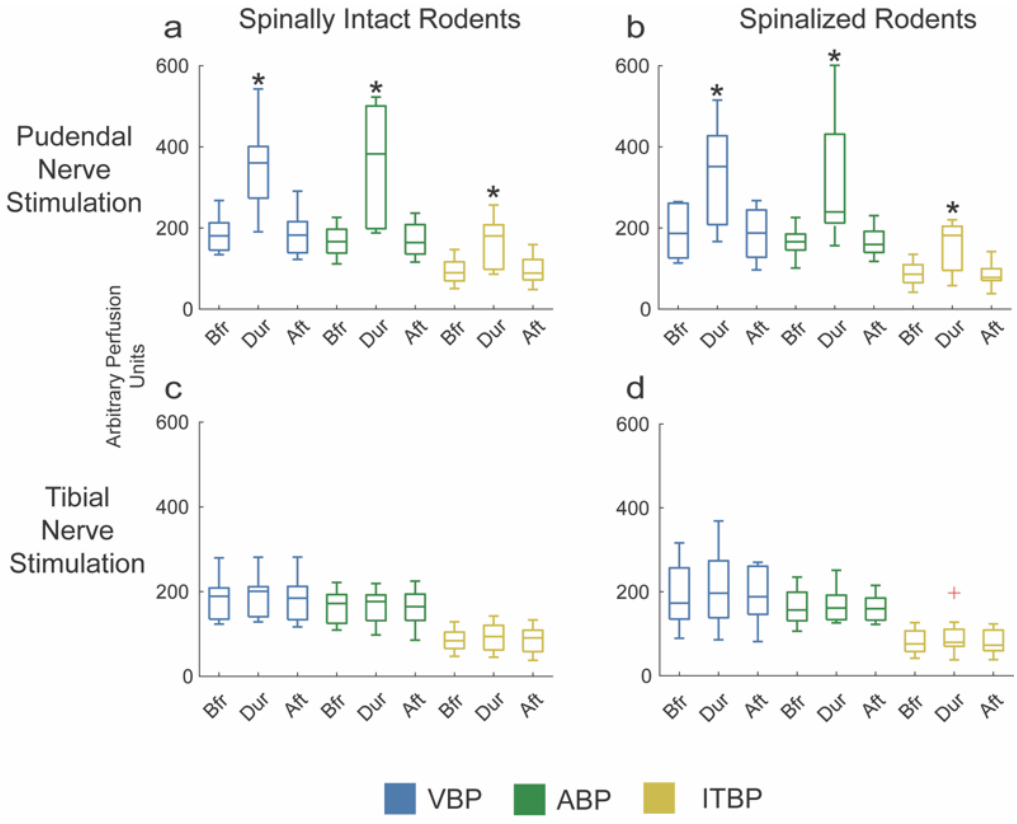

Figure 4. Boxplots of blood perfusion units across trial epochs (before, during, and after stimulation). Boxplot central lines give the median, edges indicate the interquartile ranges, whiskers represent range, and the cross denotes an outlier. Pudendal nerve stimulation trials (a, b) found elevated blood perfusion across all three ROIs during stimulation. There were no changes during tibial stimulation trials (c, d). * denotes significant difference ($p < 0.05$) between before and during stimulation epochs for an ROI.

2.4.2 Post Spinalization Recordings

Pudendal nerve stimulation trials after spinalization (Figure 3b) had an average VBP of 193 ± 61, 337 ± 131 (+74.5 ± 42.8% change from post-spinalization baseline), and 188 ± 65 APU before, during and after stimulation respectively. ABP for the same trials was 165 ± 39, 316 ± 161 (+86.6 ± 59.1%), and 167 ± 37 APU. Average ITBP was 88 ± 30, 153 ± 63 (+74.4 ± 60.7%), and 86 ± 28 APU (Figure 4b). There were no significant differences between average blood perfusion before and after spinalization for each ROI.

Tibial nerve stimulation trials had no significant differences after spinalization (Figure 3d). VBP was 193 ± 76, 211 ± 87 (+9.1 ± 11.8%), and 196 ± 65 APU prior to, during, and after stimulation. ABP was 168 ± 46, 171 ± 41 (+2.6 ± 7.8%), and 163 ± 32 APU for the same epochs. ITBP was 83 ± 29, 93 ± 45 (+10.0 ± 21.6%), and 79 ± 28 APU (Figure 4d).

2.4.3 Sampling Frequency Experiments

In the sampling frequency experiments, we sampled blood flow using the LSCI system at 1 Hz for the first 4 minutes (no stimulation) and then at 25 Hz for 2 minutes (1 minute stimulation off, 1 minute on) to capture any artifact resulting from stimulation being turned on. We then reduced the sampling rate to 1 Hz for 18 minutes during stimulation. Then, we increased the sampling rate to 25 Hz for 3 minutes (2 minutes stimulation on, 1 minute off) to capture the transition during stimulation cessation. Finally, we used a sampling rate of 1 Hz for the final 19 minutes (stimulation off). In these two experiments, we observed regular, gradual oscillations in blood perfusion for each ROI that occurred at 1–3-minute intervals and were 100 to 300 APU in amplitude (e.g., Figure 5b). These oscillations were similar in width and amplitude as occasional rapid spontaneous increases in blood flow observed in 10 trials across 4

experiments in the main cohort (e.g., Figure 5a). This similarity suggests that the rapid changes during these trials were an artifact due to the low sampling rate. The stimulation artifact period during these experiments had the same features as in other experiments, with a brief, high signal period (generally 1 second or less in length), supporting our plan of removing that period during analyses.

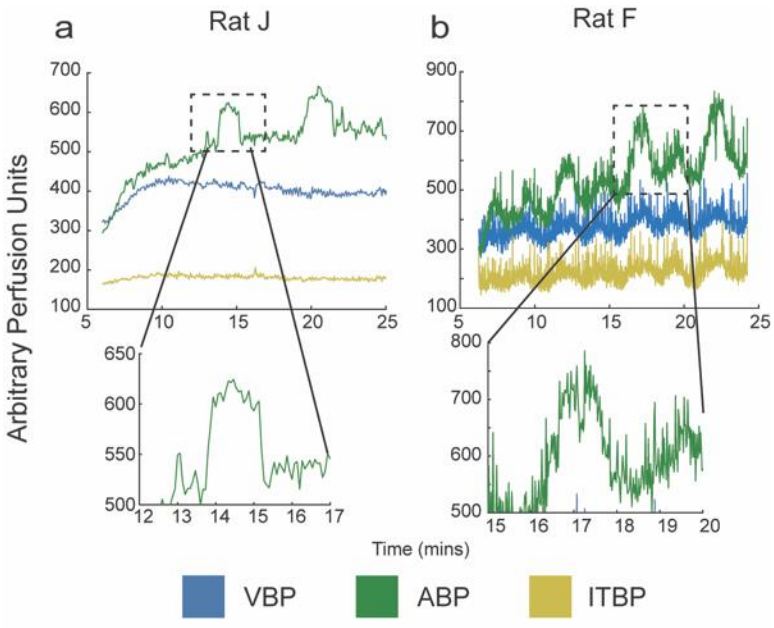

Figure 5. Intact spinal cord pudendal nerve stimulation trial periods of an experiment with abrupt increases in blood perfusion (4a) and an experiment with a higher LSCI sampling rate (4b). Top panels depict VBP, ABP, and ITBP waveforms during stimulation (0.25 and 1 Hz sampling frequency for Rat J (4a) and Rat F (4b) respectively). Bottom panels show zoomed-in plots of an individual signal oscillation in each trial of similar width (~ 2 minutes) and amplitude (150-200 APU).

2.4.4 Nerve Transection Experiments

We performed three experiments to examine the contributions of afferent and efferent pathways to the blood perfusion response to pudendal nerve stimulation. For the two pudendal nerve transection experiments with full cuts (one proximal and one distal to the stimulation location), the increases in VBP, ABP, and ITBP were abolished and there were no changes in blood perfusion for the duration of the trial. For the partial pudendal nerve transection experiment, there were still small, but noticeable increases in VBP, ABP, and ITBP during

stimulation (+35.4%, +17.0%, and +43.8% change from baseline) which returned to baseline after stimulation was ceased. After complete pudendal nerve transection, we observed no changes in blood perfusion during stimulation for any of the ROIs (+0.9%, -6.5, and +1.0% change for VBP, ABP, and ITBP respectively)

2.5 Discussion

In this study, we examined perineal blood flow changes in response to pudendal and tibial nerve stimulation. Additionally, we studied the impact of spinalization on these blood flow responses. This is the first study of its kind to use LSCI to measure perineal blood flow in animals. In intact animals, we saw a transient increase in perineal blood flow (VBP and ABP) during pudendal nerve stimulation that returned to baseline immediately after stimulation was turned off (Figure 3a). Tibial nerve stimulation in intact animals did not impact blood flow during or after stimulation was delivered (Figure 3c). After spinal cord transection, pudendal and tibial nerve stimulation had the same effect as during intact spinal cord trials (Figure 3b/d).

Perineal blood perfusion increases during pudendal nerve stimulation are likely attributable to the direct activation of motor efferents, causing the anal and urethral sphincter to contract immediately after stimulation onset[104]. This muscle activation causes a generalized increase of blood flow to the entire perineal region. Spinal cord transection (Figure 4b) had no impact on the pudendal stimulation response, excluding the involvement of a supraspinal pathway. Transecting the pudendal nerve distally abolished the response immediately, indicating that the neural pathways involved are likely direct efferents, and not a genital somato-visceral spinal reflex. Furthermore, studies demonstrating a potential genital somato-visceral reflex that increases vaginal blood perfusion have a response that persists after stimulation is turned off[34,68], which our VBP responses did not. Transecting the pudendal nerve proximally in one experiment

unexpectedly abolished the perineal blood perfusion response. It is possible that we damaged the nerve during suture placement or transection. Experimental limitations prevented a repetition of this trial. Other experimental limitations include our use of anesthesia, which may have dampened the effect of stimulation on vulvar blood flow, and the duration of stimulation. It is possible that repeated or longer stimulation sessions are required to evoke a non-direct response, increasing vulvar blood perfusion.

Prior preclinical pudendal nerve stimulation studies have used short duration (20 seconds) and long duration (30 minutes) stimulation and measured the blood perfusion response using laser doppler flowmetry (LDF) probes placed on the interior vaginal wall[34,68]. Both studies found an increase in vaginal blood flow that either began or was transiently maintained after stimulation was turned off. Vaginal blood flow gradually increased, peaked, and gradually returned to baseline during a 15-second to two-minute period. Preclinical experiments have also demonstrated the potential for tibial nerve stimulation to modulate vaginal blood flow[71,105] and that estrogen is necessary to preserve this response[105]. It is not unexpected that a balanced gonadal hormone milleu is necessary for a genital blood flow response, however little research has been done to assess the direct impact of peripheral nerve stimulation on gonadal hormones. One such study found that tibial nerve stimulation did not have an impact on serum estradiol[105], and we hypothesize that pudendal nerve stimulation may not have a direct impact on gonadal hormones either. We expected to see increases in perineal blood perfusion in our experiments, however neither pudendal nor tibial nerve stimulation had an effect on perineal blood perfusion that persisted beyond the stimulation epoch. The vagina receives its blood supply from the uterine, vaginal, and internal pudendal arteries[89]. In contrast, the perineal region, which includes the vulva and anus, is only supplied by the internal pudendal arteries[89]. It is possible that

pudendal nerve stimulation elicited changes in blood perfusion of internal tissues, such as the vagina, that could not be measured using LSCI.

Genital blood flow in women has been most commonly assessed with vaginal photoplethysmography (VPP)[93]. However, more researchers are using non-invasive techniques such as Laser Doppler Imaging (LDI)[94,96] and LSCI[46] because they report higher levels of agreement between genital arousal, subjective arousal, and lubrication. Studies using LDI or LSCI to assess genital arousal have thus far been made in clinical settings in which participants receive visual sexual stimulation. Nerve stimulation may not be sufficient to evoke a lasting vulvar blood perfusion response in an anesthetized model of genital arousal. This suggests that vulvar blood perfusion needs further investigation before it is used in anesthetized animals as a direct measurement of genital arousal.

The absence of blood perfusion changes during tibial nerve stimulation (Figure 3,4) is not entirely unexpected, as the tibial nerve innervates the lower leg and does not directly innervate the perineal region[106]. Spinal cord transection did not change the lack of response. Researchers have previously found that vaginal blood flow, measured with LDF, can increase in response to tibial nerve stimulation[71,105]. The absence of blood perfusion changes in these experiments could be attributed to a difference in recording areas (vagina vs vulva), just as in pudendal nerve stimulation trials. Clinical studies have demonstrated that tibial nerve stimulation can improve bladder dysfunction in both non-neurogenic and neurogenic populations in weekly percutaneous stimulation sessions[88,107]. In those studies, tibial nerve stimulation improved bladder storage, a function controlled by the sympathetic nervous system. However, healthy sexual functioning requires coordination of the sympathetic, parasympathetic, and somatic nervous systems and it is the parasympathetic pathways that cause smooth muscle relaxation and vaginal vasocongestion.

Different stimulation parameters or repeated stimulation sessions (as is common for clinical treatment) may cause a change in VBP similar to perfusion changes observed in vaginal tissue.

We did not expect to see changes in ITBP during pudendal nerve stimulation, as the pudendal nerve does not directly innervate the superficial regions of the inner thigh. Spinalization did not have an impact on the average ITBP, across all stimulation epochs (Figure 4). Distal pudendal nerve transection abolished the increase in ITBP observed in intact animals during pudendal nerve stimulation. The pudendal nerve does not directly innervate any regions of the leg, but one cadaver study discovered that the sciatic artery can arise from the internal pudendal artery[108]. It is possible that activation of motor efferents caused blood flow to increase in pudendal arteries that then branch off and supply the inner thigh. Thus, the increases in ITBP are likely attributable to general blood flow to the region due to the contraction of the pelvic floor muscles placing an increased demand on blood supply, further supporting our conclusion that the increases in vulvar blood perfusion are due to somatic activation of the pelvic floor musculature.

2.6 Conclusion

This study sought to assess the impact of two potential treatment modalities for FSD (pudendal and tibial nerve stimulation) on perineal blood perfusion. We theorize that improvements in FSD symptoms are related to improvements in genital arousal and that it is possible to modulate genital arousal via pudendal or tibial nerve stimulation. Both treatment modalities have demonstrated potential in treating FSD. The genital nerve, a distal branching of the pudendal nerve, is a promising target because it can be accessed superficially, and stimulation has shown to modulate sexual function[67]. Tibial nerve stimulation is ideal for similar reasons as it is both superficial and easy to access, but more work is necessary to understand how

it modulates genital blood flow. However, this preliminary study did not find any lasting changes in genital arousal as measured by perineal blood perfusion. We suspect that this contrast with prior preclinical studies is due to a difference in blood perfusion location and that the responses we did observe were due to muscle contractions requiring an increased blood supply to the region. An improved animal model for evaluating genital blood perfusion would incorporate simultaneous measurements of vaginal and vulvar blood perfusion. This model is necessary to understand the complete hemodynamics of different genital structures, in particular the relationship between vaginal and vulvar blood perfusion during arousal.

Chapter 3 Acute Genital Nerve Stimulation Increases Subjective Arousal in Women with and without Spinal Cord Injury

(*Submitted for publication*[109])

3.1 Abstract

Introduction: Female sexual dysfunction is a potentially life-altering condition that impacts an estimated 40% of women. Unfortunately, female sexual function has been historically understudied which has led to limited treatment options for sexual dysfunction. These treatment options have focused on improving low desire as opposed to more physiological aspects of sexual function, such as arousal or lubrication. Neuromodulation has demonstrated some success in improving female sexual dysfunction symptoms. We developed a pilot study to investigate the short-term effect of electrical stimulation of the genital nerve and tibial nerve on sexual arousal in healthy women, women with female sexual dysfunction, and women with spinal cord injury and female sexual dysfunction.

Methods: This study consists of a randomized crossover design in three groups: women with spinal cord injury, women with non-neurogenic female sexual dysfunction, and women without female sexual dysfunction or spinal cord injury. The primary outcome measure was change in vaginal pulse amplitude from baseline. Secondary outcome measures were changes in subjective arousal, heart rate, and mean arterial pressure from baseline. Participants attended one or two study sessions where they received either transcutaneous genital nerve stimulation or tibial nerve stimulation. At each session, a vaginal photoplethysmography sensor was used to measure vaginal pulse amplitude during a 5-minute baseline, 20 minutes of nerve stimulation, and a 5-

minute recovery period. Participants also rated their level of subjective arousal at four timepoints during the testing session and were asked to report any pelvic sensations.

Results: We found that subjective arousal increased significantly from before to after stimulation in genital nerve stimulation study sessions across all women. Tibial nerve stimulation had no effect on subjective arousal. There were significant differences in vaginal pulse amplitude between baseline and stimulation, baseline and recovery, and stimulation and recovery periods among participants, but there were no trends across groups or stimulation type. Two participants with complete spinal cord injuries experienced genital sensations.

Discussion: This is the first study to measure sexual arousal in response to acute neuromodulation in women. This study demonstrates that genital nerve stimulation, but not tibial nerve stimulation, can increase subjective arousal, but the effect of stimulation on genital arousal is inconclusive. This study provides further support for genital nerve stimulation as a treatment for female sexual dysfunction. Studies on the physiological effect of repeated stimulation sessions are needed to further examine the sexual health benefits of neuromodulation.

Conclusion: We observed a significant increase in subjective arousal during genital nerve stimulation but not tibial nerve stimulation across patients, and varying effects on VPA across stimulation sessions and patient groups.

3.2 Introduction

Female sexual function has been historically understudied, leading to limited treatment options for the approximately 40-50% of women who suffer from symptoms associated with female sexual dysfunction (FSD)[110]. Existing treatment options for FSD, such as bremelanotide[49] and flibanserin[111], primarily target hypoactive sexual desire disorder (HSDD). There is a lack of treatments that target challenges with the physiological aspects of sexual function, such as

lubrication or arousal. Sildenafil, a successful pharmaceutical for treating male sexual arousal dysfunction, was pursued for FSD but ultimately abandoned due to its low efficacy rate and high incidence of adverse events[83].

FSD can have a variety of etiologies, one of which is spinal cord injury (SCI). People with SCI report sexual function as one of their top priorities to regain[92,112] and sexual function is an important factor in quality of life for all adults[78]. Location and severity of the injury often determine which aspects of sexual function are impacted (e.g., arousal, desire). Psychogenic, but not reflexogenic, arousal is often possible in women with sacral level injuries while reflexogenic arousal is generally retained in women with injuries above the lumbar level[37]. These two examples demonstrate the heterogeneity in FSD symptoms among women with SCI, an underserved population that would particularly benefit from FSD treatments developed with pathophysiology taken into consideration.

Neuromodulation, or electrical stimulation of neural targets, has shown some promise in treating FSD in non-neurogenic women. Clinical trials using sacral neuromodulation to treat women with bladder dysfunction found that their sexual function, as evaluated by the female sexual function index (FSFI)[43], improved as an unanticipated benefit[113–115]. Other bladder dysfunction neuromodulation targets have been investigated as treatments for FSD, including tibial nerve stimulation (TNS)[66,67] and genital nerve stimulation (GNS)[67]. Although the mechanisms of these interventions are not fully understood, we theorize that GNS and TNS can improve FSD by increasing genital arousal. Genital arousal, often measured by vaginal blood flow, has shown to have short-term increases during peripheral nerve stimulation in preclinical models. Animal studies using TNS have shown increases in vaginal blood flow[71,105] and we hypothesize that the underlying mechanisms involve a spinal reflex pathway. Similarly, animal

studies using pudendal nerve stimulation, the proximal source of the genital nerve, have shown increases in vaginal blood flow[34,68]. These studies suggest that pudendal nerve stimulation activates spinal pathways that in turn activate the pelvic efferents that modulate vaginal blood flow. We hypothesize that increased blood flow contributes, at least in part, to improved FSFI scores for women with FSD.

Clinical studies on female sexual function often measure genital arousal, however this is the first study to measure genital arousal in response to transcutaneous neuromodulation. We sought to investigate if neuromodulation can modulate vaginal blood flow in women with SCI, able-bodied women with non-neurogenic FSD, and able-bodied women without FSD as healthy controls. Our goal was to assess if short-term transcutaneous electrical stimulation of the genital or tibial nerve can modulate genital and subjective arousal. We chose these three groups of participants to assess which treatments were best at evoking a blood flow response and subjective arousal given the presence of SCI or FSD.

3.3 Methods

All study activities were approved by the University of Michigan Institutional Review Board (HUM00148746) prior to initiation and all data was collected at Michigan Medicine between November 2020 and March 2022. We recruited participants via physician referral, flyers placed in relevant clinics in the local area, and online through a University of Michigan health research portal. The study is registered at clinicaltrials.gov under identifier NCT04384172.

This study consists of a randomized crossover design with three groups: women with SCI (SCI), women with non-neurogenic FSD (FSD), and women with No Dysfunction and who are Able-Bodied (NDAB). Participants were screened for eligibility with a clinical study coordinator prior to enrollment. All participants were over 18 years old, biologically female, and sexually

active at least once a month. SCI participants could be interested in sexual activity if not sexually active. To be included in the SCI arm, participants had to have a clinically diagnosed spinal cord injury at grade AIS (American Spinal Injury Association Impairment Scale) A-C at a level within C6-S1 at least six months prior to enrollment and a short-form FSFI[116] score below 19. Women with FSD were neurologically intact with a short-form FSFI score below 19 and an FSFI lubrication sub-score below or equal to 3. Women without FSD were neurologically intact with a short-form FSFI score above or equal to 19 and FSFI lubrication sub-score above 4. Exclusion criteria for all participants were as follows: (1) pregnant, (2) clinically diagnosed bladder dysfunction, pelvic pain, or other pelvic organ symptoms, (3) active infection or active pressure sores in the perineal region, (4) epilepsy, and (5) implanted pacemaker or defibrillator. Additional exclusion criteria for SCI participants included worsening in motor or sensory function in the last month. NDAB and FSD participants were also excluded if they had clinically diagnosed bladder dysfunction, pelvic pain, or other pelvic organ symptom.

After obtaining consent, we instructed participants to submit demographic information and complete five clinically validated surveys: the American Urological Association Symptom Index (AUASI) bladder symptom index[117], the female sexual function index (FSFI)[43], the fecal incontinence severity index (FISI)[118], the patient assessment of constipation-symptoms (PAC-SYM)[119], and the short-form qualify of life survey (SF-36)[120]. The surveys were collected online in REDCap, a standard clinical tool for survey data collection[121]. Participants completed one or two study sessions corresponding to two stimulation targets: the genital nerve and tibial nerve. We used block randomization, with block sizes of 10 for each group, to determine which nerve target was used in the first study session. Study team members and participants were not blinded. Participant's second study session was one to five months after their first. Participants filled out

pelvic function surveys that asked them about their bladder, bowel, and sexual function for a given day. The surveys were filled out daily from two days prior to two days after each study session to monitor any carryover effects from neuromodulation.

At each study session, participants were asked to sit, partially reclined in a comfortable position. A vaginal photoplethysmography transducer (TSD204A, Biopac Systems Inc., Goleta, CA) was placed in the vaginal canal to monitor vaginal pulse amplitude (VPA), a measurement of relative vaginal blood flow[122]. A clinician placed two round surface electrodes (1.25 inch diameter, ValuTrode Neurostimulation Electrodes CF3200, Axelgaard Manufacturing Co. Ltd., Fallbrook, CA) on either side of the clitoris[123] for GNS study sessions and above the malleolus and on the bottom of the foot[124] for TNS study sessions. Stimulation was delivered with a transcutaneous electrical nerve stimulation (TENS) device (Empi Select 199584, Medi-Stim Inc., Wabasha, MN, USA). The amplitude was determined by slowly increasing it from 0 mA until a maximum comfortable level or 60 mA was reached, whichever was lower. We recorded VPA at a sampling rate of 200 Hz during a 5-minute baseline period, 20 minutes of 20 Hz nerve stimulation at the pre-determined amplitude, and a 5-minute post-stimulation period for a total of 30 minutes. We asked participants to rate their level of subjective arousal on a 5-point Likert-style scale at four times throughout the recording trial: before baseline, before stimulation, after stimulation, and after the washout period. After the trial, we asked participants their opinion of the TENS device, if it elicited any genital sensations, and if they would consider using it.

All data analysis was performed in MATLAB (Mathworks, Natick, MA, USA). VPA signals for each participant session were processed before subsequent analysis and statistics across participants. We bandpass filtered the raw VPA signal from 0.5 to 30 Hz and identified peaks and troughs using MATLAB's findpeaks function. We visually inspected the peaks and

troughs for artifact removal. We removed obvious artifacts if they did not conform to the typical sawtooth shape[125] or had an trough-to-peak amplitude that had over a 100% increase from the previous waveform[126]. The average percentage of data points removed was 14.3%. We calculated trough to peak amplitude and binned the data into 10 second intervals[127]. Binned values were averaged for three time periods: 5-minute baseline ($VPA_{Baseline}$), 20-minutes of stimulation (VPA_{Stim}), and 5-minute recovery ($VPA_{Recovery}$). We made comparisons between these three periods within each participant with a one-way Analysis of variance (ANOVA) followed by post-hoc pairwise Tukey HSD tests. We calculated the percent change for each participant between each of $VPA_{Baseline}$, VPA_{Stim}, and $VPA_{Recovery}$, and made comparisons across participants for VPA_{Change} ($VPA_{Stim} - VPA_{Baseline}$) for each stimulation location with a paired t-test. We compared subjective arousal scores between each timepoint with a paired t-test. We compared baseline heart rate and mean arterial blood pressure to the last heart rate and blood pressure recorded during stimulation with paired t-tests. We compared the survey scores (SF-36, AUASI, PAC-SYM, FISI, and FSFI) across participants from the three different groups with pairwise t-tests. All statistical analysis used alpha = 0.05 to determine significance.

3.4 Results

We screened 101 participants for eligibility over the phone. Of the 29 participants screened for the NDAB group, 20 were excluded for not meeting inclusion criteria, 3 declined to participate, and 3 were withdrawn before they could complete a single study session due to communication challenges. Of the 28 participants screened for the FSD group, 24 did not meet the inclusion criteria and 1 participant was lost to follow up prior to any sessions. Of the 44 participants evaluated for the SCI group, 36 did not meet the inclusion criteria and 5 declined to participate. This resulted in 3 participants in each group that completed at least one study

session. One participant was lost to follow up in each of the NDAB and FSD groups after completing their first study session. In the SCI group, SCI-1 was lost to follow up after her first session and SCI-3 became ineligible after her first session. Demographics for all three groups of participants can be found in Table 2.

Table 2. Participant demographics

Participant ID	Age	Height (m)	Weight (kg)	Race	Ethnicity
NDAB-1	22	1.60	64	Asia or Pacific Islander	Non- Hispanic or Latino
NDAB-2	25	1.57	81	White, Caucasian	Non- Hispanic or Latino
NDAB-3	27	1.52	77	Multiracial	Hispanic or Latino
SCI-1 (sacral-level spina bifida presents like SCI)	49	1.42	57	White, Caucasian	Non- Hispanic or Latino
SCI-2 (T5, AIS-A, 23 months post-injury)	47	1.60	68	White, Caucasian	Non- Hispanic or Latino
SCI-3 (T2, AIS-A, 15 years post-injury)	36	1.60	66	White, Caucasian	Non- Hispanic or Latino
FSD-1	33	1.70	59	White, Caucasian	Non- Hispanic or Latino
FSD-2	25	1.68	75	Multiracial	Non- Hispanic or Latino
FSD-3	31	1.65	48	White, Caucasian	Non- Hispanic or Latino

Survey results averaged across each participant group can be found in Table 3. The SCI group and FSD group reported lower FSFI lubrication sub-scores than the NDAB group ($p < 0.05$). The FSD group reported significantly lower total FSFI scores than both NDAB and SCI groups ($p < 0.005$ and $p < 0.05$). All other survey scores were not significantly different between participant groups. The average stimulation amplitude for GNS and TNS sessions was 28.8 ± 26.8 mA and 33.4 ± 24.4 mA respectively.

Table 3. Participant survey results

Participant ID	SF36 (0 to 100[+])	AUASI (35 to 0[+])	PACSYM (48 to 0[+])	FISI (61 to 0[+])	FSFI[%] (2 to 36[+])	FSFI Lubrication (1 to 6[+])
NDAB-1	76.9	2	2	0	32.5	6
NDAB-2	69.2	1	11	0	33.9	6
NDAB-3	53	5	0	3	23.5[$]	6
SCI-1	35.5	8	10	13	22.7	3.9
SCI-2	53.4	2	6	19	15.4	1.2
SCI-3	52.6	2	3	0	21.8	1.8
FSD-1	74.6	0	0	0	12	1.2
FSD-2	53.8	5	14	21	7.7	1.2
FSD-3	57.5	0	0	0	9.8	3
Average NDAB	66.4	2.7	4.3	1.0	30.0	6.0
Average SCI	47.2	4.0	6.3	10.7	20.0	2.3*
Average FSD	62.0	1.7	4.7	7.0	9.8*	1.8*

* $p < 0.05$ compared to NDAB group
+ indicates "no dysfunction" score
% clinical cutoff score for dysfunction = 26.55[45]
$ low score due to temporary sexual inactivity after initial screening with short-form FSFI

In GNS trials, we found significant increases in subjective arousal from before the trial to after the stimulation period ($p < 0.05$) across all participants (Figure 6a). These changes were also significant ($p < 0.05$) from before the trial until after the washout period. Participants in each of the three groups reported increased arousal during GNS. There were no significant changes in subjective arousal across TNS trials (Figure 6b). There were no significant differences between heart rate or mean arterial blood pressure between baseline and stimulation in GNS or TNS trials. The daily pelvic function surveys indicated that most participant's bladder, bowel, and sexual function were stable and no participants reported carry-over effects from the stimulation session.

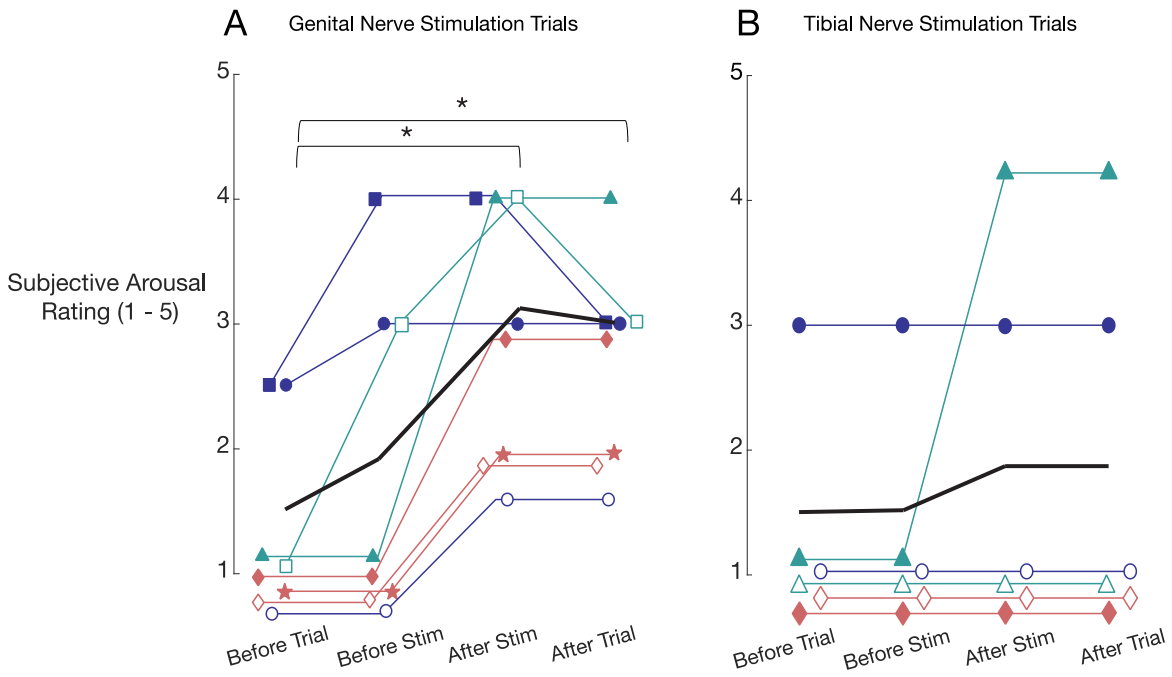

Figure 6. Subjective arousal scores for GNS and TNS sessions. Icons represent the same individual participants. Icon color corresponds to group (indigo = able bodied, non-dysfunction, teal = SCI, rose = FSD). Solid black line denotes average values. Subjective arousal increased significantly ($p < 0.05$) in GNS trials, but not TNS trials.

Most participants reported genital sensations in response to GNS (7/8). Comments about receiving GNS were mostly neutral and participants indicated that they would be willing to use a TENS device at the genital nerve. Half of participants (3/6) reported genital sensations during TNS and seemed just as willing to use TENS at the tibial nerve if they knew it would help with their sexual dysfunction. Table 4 contains all participant feedback.

Table 4. Paraphrased participant responses to session follow up questions.

Participant ID	Participant comments about GNS			Participant comments about TNS		
	What is your general opinion of the device?	Did it elicit any genital arousal responses (e.g. lubrication, tingling, pleasurable sensations)?	Would you consider further use of the device?	What is your general opinion of the device?	Did it elicit any genital arousal responses (e.g. lubrication, tingling, pleasurable sensations)?	Would you consider further use of the device?
NDAB-1	Not painful	Lubrication and tingling	Yes	No discomfort, fine	Maybe lubrication	If dysfunction
NDAB-2	Weird, kind of strange	Maybe lubrication towards the end of the stimulation	NA*	Numbing	No	No
NDAB-3	Neutral, kind of strange	A little tingling	If had dysfunction	Lost to follow up		
SCI-1	Fine, comfortable	Lubrication, tingling, a little bit of pleasurable sensations	Yes	Lost to follow up		
SCI-2	It works, nothing uncomfortable about it. Has to concentrate to feel things	Tingling and pulsation	Yes	Easy at home, comfortable	Tingling and throbbing	Yes
SCI-3	Withdrawn after becoming ineligible			Would use the device	Tingling and bladder spasms	Yes
FSD-1	Neutral	Tingling	Yes	Neutral, ambivalent	No	Yes
FSD-2	Pretty neutral	None, just felt like tapping	Might in a non-clinical setting	Fine	No	Potentially
FSD-3	More warmth than arousal	Warmth and blood flow, but no arousal	Probably not	Lost to follow up		

* NA denotes “Not Applicable” as participant misunderstood the question

VPA recordings from one participant in the NDAB group had corrupted data from both of their study sessions. All participants except one (FSD-3) had significant differences in their peak-to-peak VPA between at least two time periods in both GNS and TNS sessions. These changes were not consistent within or across groups. Across GNS sessions, two participants had an overall increase from $VPA_{Baseline}$ to $VPA_{Recovery}$, four had an overall decrease, and one participant had no changes (Figure 7). Across TNS sessions, 3 participants had an overall increase from $VPA_{Baseline}$ to $VPA_{Recovery}$, 1 participant had an overall decrease, and 1 participant had a decrease from $VPA_{Baseline}$ to VPA_{Stim} that returned to baseline values in $VPA_{Recovery}$ (Figure 8). The average VPA_{Change} was $+3.5 \pm 26.7\%$ and $+3.5 \pm 14.0\%$ for GNS and TNS sessions respectively, which were not significantly different from zero. Average $VPA_{Baseline}$, VPA_{Stim}, and $VPA_{Recovery}$, as well as percent changes between each of the time periods for each participant, can be found in Tables 5 & 6. Subjective arousal and VPA data are publicly accessible online[128].

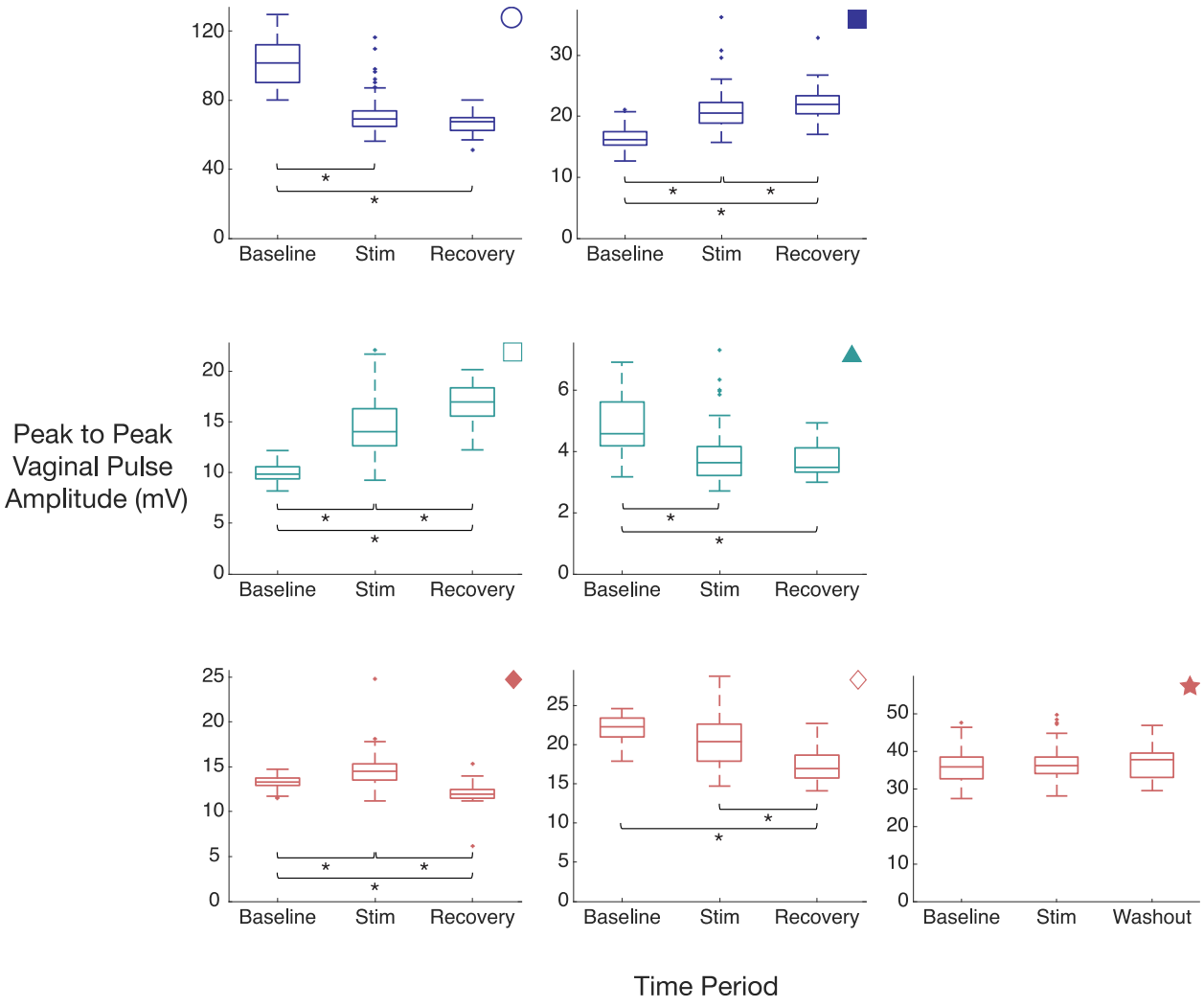

Figure 7. Boxplots of peak-to-peak VPA across different time periods (Baseline, Stim, and Recovery) in GNS sessions. Boxplot central lines give the median, edges indicate the interquartile range, and dots represent outliers. Color and icons correspond to participants and groups as per Figure 6. * denotes significant difference ($p < 0.05$).

Table 5. Summary of VPA mean values (arbitrary units) during transcutaneous GNS trials.

Subject	Baseline (mean ± se)	Stimulation (mean ± se)	Recovery (mean ± se)	Baseline to Stimulation (%)	Baseline to Recovery (%)	Stimulation to Recovery (%)
NDAB-2	102.2 ± 2.7	70.6 ± 0.9	66.9 ± 1.1	-30.9	-34.5	-5.3
NDAB-3	16.4 ± 0.4	20.8 ± 0.3	22.2 ± 0.5	+26.5	+35.5	+7.1
SCI-1	10.0 ± 0.2	14.5 ± 0.3	17.0 ± 0.4	+45.5	+70.7	+17.4
SCI-2	4.9 ± 0.2	3.8 ± 0.1	3.7 ± 0.1	-22.2	-24.0	-2.4
FSD-1	13.2 ± 0.2	14.6 ± 0.2	12.0 ± 0.3	+10.4	-9.4	-17.9
FSD-2	22.0 ± 0.3	20.6 ± 0.3	17.5 ± 0.4	-6.2	-20.4	-15.1
FSD-3	36.1 ± 0.9	36.7 ± 0.4	37.6 ± 0.9	+1.7	+4.1	+2.4
Average	29.2 ± 33.7	25.9 ± 22.1	25.3 ± 21.2	+3.5 ± 26.7	+3.1 ± 37.6	-2.0 ± 12.3

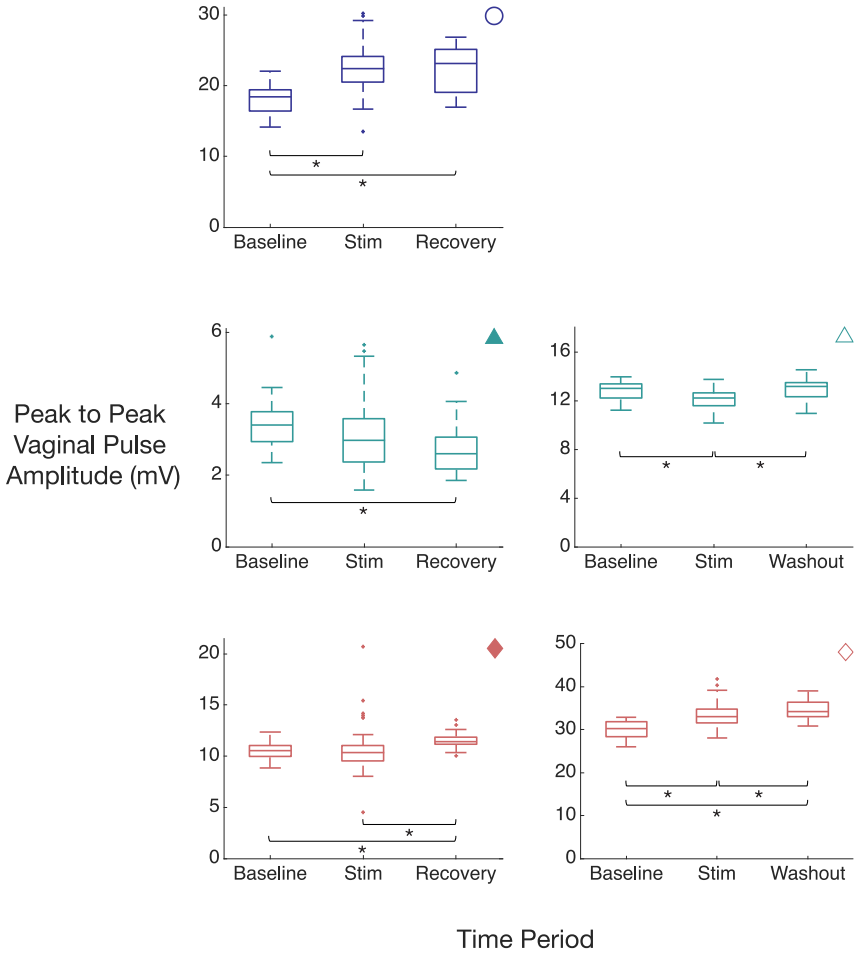

Figure 8. *Boxplots of peak-to-peak VPA across different time periods (Baseline, Stim, and Recovery) in TNS sessions. Color and icons correspond to participants and groups as per Figure 6. * denotes significant difference (p < 0.05).*

Table 6. Summary of VPA mean values (arbitrary units) during transcutaneous TNS trials.

Subject	Baseline (mean ± se)	Stimulation (mean ± se)	Recovery (mean ± se)	Baseline to Stimulation (%)	Baseline to Recovery (%)	Stimulation to Recovery (%)
NDAB-2	18.1 ± 0.4	22.5 ± 0.3	22.4 ± 0.6	+24.3	+23.7	-0.5
SCI-2	3.5 ± 0.1	3.1 ± 0.1	2.7 ± 0.7	-11.1	-21.0	-11.1
SCI-3	12.8 ± 0.2	12.1 ± 0.1	13.0 ± 0.1	-5.0	+1.6	+7.0
FSD-1	10.5 ± 0.2	10.4 ± 0.2	11.6 ± 0.1	-0.9	+9.9	+10.8
FSD-2	30.2 ± 0.4	33.3 ± 0.2	34.6 ± 0.4	+10.4	+14.6	+3.8
Average	15.0 ± 10.0	16.3 ± 11.8	16.8 ± 12.1	+3.5 ± 14.0	+5.7 ± 17.0	+2.0 ± 8.4

3.5 Discussion

In this study, we sought to understand the effect of one-time transcutaneous GNS and TNS on genital arousal, measured as vaginal pulse amplitude. We also investigated the effect of GNS and TNS on subjective arousal. This is the first study of its kind to measure genital arousal in response to neuromodulation in women. We observed a significant increase in subjective arousal during GNS but not TNS across patients, and varying effects on VPA across stimulation sessions and patient groups. Women in all three participant groups gave positive comments to receiving neuromodulation and women with complete SCI experienced genital sensations in response to GNS and TNS.

Subjective arousal increased following stimulation across all groups of participants in GNS sessions. As expected, the FSD group had lower subjective arousal than their NDAB and SCI counterparts after stimulation. Sexual function involves complex coordination of the parasympathetic, sympathetic, and central nervous system. There are several different proposed models of the sexual response cycle[20,129,130]. Most models recognize that deficits in physiological areas (e.g., arousal) can influence the psychological (e.g., desire), making it difficult to pin-point

the reason for lower subjective arousal in the participants with FSD. It is possible that the awareness of their FSD status in an isolated, clinical setting made them feel uncomfortable and led to lower subjective arousal. Participants with FSD experienced the fewest genital sensations and one participant (FSD-2) indicated that they might feel more receptive to using GNS in a non-clinical setting (Table 4). Unexpectedly, some participants in GNS sessions (3/8) had increases in subjective arousal before stimulation was turned on. This could perhaps be due to the presence of the VPA probe or anticipation of increased arousal.

All participants except one had significant changes in their peak-to-peak VPA between at least two periods (baseline, stim, or recovery) (Figure 7, 8). Three participants (NDAB-3, FSD-1, and FSD-2) had opposing trends in their peak-to-peak VPA between GNS and TNS sessions. It may be that not all women are responders to GNS or TNS, as is common for neuromodulation therapies[131–133]. There were no consistent VPA trends (decrease, increase, no change) within groups, which is not entirely unexpected given the small sample size. However, we did hypothesize that GNS would increase VPA based on prior animal studies receiving stimulation at the pudendal nerve, the main trunk of the genital nerve. Cai et al[34] theorized that pudendal nerve stimulation activates a spinal autonomic efferents via the pelvic nerve. Larger studies are needed to examine these trends further. For example, in women with SCI, it could be that one nerve target is more effective than the other depending on their injury level and severity.

As participants were not exposed to sexual stimuli during the study procedure, our VPA results are not directly comparable with prior VPA studies. It is possible that repeated stimulation sessions over time, as is common for percutaneous tibial nerve stimulation for bladder dysfunction[63], may yield consistent VPA changes as was found in a prior study that

found repeated stimulation can increase FSFI scores[67]. Although animal studies have reported genital blood flow increases in response to tibial or pudendal nerve stimulation[34,68,71,77,105], those studies used anesthetized animals and directly stimulated the nerve, which limits direct comparisons to this study. An awake animal model study, such as the one by Zimmerman et. al in which they found increased sexual receptivity after 6 weeks of biweekly TNS[134], would provide a more analogous study paradigm to clinical studies.

Subjective arousal increased for one participant (SCI-2) in TNS sessions. It is likely that the mechanisms of TNS to modulate sexual function involve an indirect pathway to the pelvic organs, similar to the spinal reflexes proposed for bladder function[64]. Notably, this participant has a complete SCI (AIS-A) and reports that she does not experience any sensation below her level of injury (T5). It is possible that there are residual fibers in her spinal cord that are carrying afferents that were activated by GNS or TNS. Another hypothesis is that afferents from the genitals during arousal, activated by GNS or TNS, could be circumventing the spinal cord via the vagus nerve, leading to subjective arousal. One study in women with complete SCI asked participants to perform vaginal-cervical self-stimulation in a functional magnetic resonance imaging machine. Researchers found that the nucleus solitary tract (NTS), where the vagus nerve projects in the brain stem, was active during self-stimulation[135]. This is supported by an animal study that found neurons in the NTS that responded to vaginal distension and cervical stimulation[136].

Almost all women were willing to use GNS or TNS outside of this study, provided it would help with their sexual dysfunction (if they had it). There was more hesitancy with GNS as a therapy among the FSD group, perhaps due to the sensitive location of the electrodes. All

participants who were lost to follow up after their first study session received GNS, which may suggest that the method for delivering GNS could be improved. Both nerve targets have distinct advantages. GNS was able to increase subjective arousal but is applied at a sensitive location. Women may find they are more comfortable with stimulation at their ankle rather than their genitals and TNS is easier to administer at the ankle, however the mechanisms of how it improves sexual function are less clear.

Our study did not include any audio-visual materials, so we are limited in comparing our results to clinical studies that include videos with their interventions[137]. We are also limited by our sample size (n = 3 per group), which prevented us from identifying trends within or across participants groups. We have now established a baseline for VPA response to GNS and TNS in a one-time session, and although there were no trends in VPA across participants, individual participants had a VPA_{Change} as large as +70.7%. It is likely that the clinical environment with study team members present coupled with the lack of sexual stimuli contributed to an inhibition of sexual arousal, dampening genital arousal responses.

3.6 Conclusion

To our knowledge, this is the first clinical study to measure subjective and genital arousal in response to neuromodulation. We sought to understand the impact of two potential treatment modalities, transcutaneous GNS and TNS, on subjective arousal and vaginal pulse amplitude during a period of nerve stimulation. We found that GNS, but not TNS, increased subjective arousal across all participants. We did not observe a consistent VPA response to GNS or TNS across all participants or within participant groups. All SCI participants experienced genital sensations during GNS and TNS sessions. Future studies may incorporate audio-visual materials

or another type of sexual stimuli to better facilitate arousal. Studies with repeated stimulation sessions over time may find more clinically relevant improvements in sexual function.

Chapter 4 The Effect of Transcutaneous Genital and Tibial Neuromodulation on Gynecological Hemodynamics during Visual Erotic Stimuli

4.1 Abstract

Introduction: Sexual dysfunction affects approximately 22-43% of women. Unfortunately, there are few treatments for people with gynecological dysfunctions. Genital and tibial neuromodulation have been investigated as a treatment for female sexual dysfunction (FSD). We developed a study to investigate if transcutaneous genital or tibial nerve stimulation could modulate the genital arousal blood flow response to erotic stimuli in three cohorts: women with spinal cord injury (SCI), women with FSD, and healthy controls.

Methods: This study is a randomized crossover design. The primary outcome measures were the change in vaginal pulse amplitude from the first erotic video segment (stim off) to the second (stim on). The second outcome measures were the change in subjective arousal, heart rate, and mean arterial pressure from the first to the second erotic video. Participants attended one or two study sessions and received 20 minutes of transcutaneous genital or tibial neuromodulation while watching a sequence of neutral and erotic videos. At each session, genital arousal was measured with a vaginal photoplethysmography sensor. Participants recorded their video preference and were asked about their willingness to use the stimulation device.

Results: We found that FSD participants had higher VPA responses to the erotic videos than healthy controls, both with and without stimulation. TNS more consistently led to higher VPA

responses than GNS. There was a low level of sexual concordance across the healthy controls and women with SCI, but not the FSD cohort.

Discussion: This study demonstrates the potential of GNS and TNS as a therapy for FSD, but many confounding factors need to be unraveled first. A larger study, with a randomized video and stimulation sequence would provide further insights into genital and tibial neuromodulations potential to treat FSD.

4.2 Introduction

Sexual function is a frequently overlooked part of healthcare, especially for people with gynecological anatomy. For example, there is a discrepancy between how informed women and men are about the side effects of brachytherapy, often used for cervical or prostate cancer, on sexual function[138]. Sexual healthcare inequities exist for several reasons, a major one being the historical funding and execution of pharmaceutical and biomedical research from a predominately male perspective. This may explain why there are few treatment options available for gynecological dysfunctions, even though roughly 22-43% of women have poor sexual functioning according to a a study using the Female Sexual Distress Scale, a self-reported survey[139].

Women with neurological injuries such as spinal cord injury (SCI), often have impaired sexual functioning and report it as one of their top priorities to restore[3]. The likelihood of impairment in a specific facet of sexual arousal (e.g. orgasm) for women with SCI correlates

with the level and area of injury[140]. For example, psychogenic arousal is more likely to be preserved if the T11 – L2 region is undamaged, and orgasm is typically preserved if the sacral arc is intact. Women with non-neurogenic and neurogenic female sexual dysfunction (FSD) have limited treatment options despite sexual health being associated with mental health, physical health, and overall well-being[141].

There are some pharmaceuticals for female sexual dysfunction, such as flibanserin and bremelanotide, which are approved for hypoactive sexual desire disorder[142]. These drugs act on the central nervous system and can cause adverse side effects, primarily nausea[143,144]. Bremelanotide is administered "on-demand" by the user, via an injection prior to sexual activity. Another pharmaceutical, sildenafil, the popular male erectile dysfunction medication, has been investigated and shown some promise in improving genital arousal, but almost half of the women who took the drug experienced headaches, in addition to other adverse events[52]. There is a need for an easily administered treatment that improves genital arousal with minimal negative side effects.

One of the primary challenges in studying genital arousal is the lack of clinically relevant quantitative metrics for people with gynecological anatomy. Vaginal pulse amplitude (VPA) is measured with a vaginal photoplethysmography transducer and is the most common metric for genital arousal because of the importance of blood flow in the sex response cycle. However, changes in VPA have a low sexual concordance, also known as subjective-genital arousal desynchrony[145], which brings up the question of its utility in sexual health research. We need a

biomarker of genital arousal that has higher sexual concordance. This may assist in assessing the extent of someone's sexual problems, providing more detailed information as to the facets of sexual function that are impaired, which may lead to improved, targeted treatment options for generalized FSD.

Neuromodulation, or targeted stimulation of a neural target, is a potential therapy for FSD. Two minimally invasive nerve targets with the potential to treat FSD are the genital and tibial nerves. Animal studies have demonstrated that stimulation of the pudendal nerve, which branches into the genital nerve, and the tibial nerve can modulate genital blood flow[10–13]. Repeated percutaneous tibial nerve stimulation (TNS) is routinely used for bladder incontinence[65] and transcutaneous TNS has since been investigated as a treatment for FSD[146]. Genital nerve stimulation (GNS) has also been investigated as a treatment for pelvic organ dysfunctions such as urinary dysfunction or bowel incontinence[147,148], but there are comparatively fewer studies on sexual function. In our preceding study[109], as described in Chapter 3, we investigated the effect of acute transcutaneous GNS and TNS on vaginal blood flow measured with vaginal photoplethysmography[11]. We found that GNS may increase subjective arousal and mixed effects of GNS and TNS on VPA.

We believe that the presence of the study team members in the clinical testing room as well as the absence of erotic videos may have obscured the VPA data in our prior study. The brainstem has descending inhibitory pathways that prevent sexual arousal in environments that are inappropriate to the individual. Erotic videos have been used in the field of sexology for

decades[149,150] to overcome brainstem inhibition in clinical settings (e.g. hospital room). In this study, we recorded VPA and subjective arousal ratings while each participant watched a series of neutral and erotic videos, with and without GNS or TNS. We hypothesize that the addition of erotic stimuli will allow us to make more meaningful inferences about the effect of stimulation on genital arousal.

4.3 Methods

All study activities were approved by the University of Michigan Institutional Review Board (HUM00148746) prior to initiation and all data was collected at Michigan Medicine between September 2022 and May 2023. We recruited participants via physician referral, flyers placed in relevant clinics in the local area, and online through a University of Michigan health research portal. The study is registered at clinicaltrials.gov under identifier NCT04384172. This study was a randomized crossover design in three cohorts: women with SCI, women with FSD, and women as healthy control termed Non-Dysfunction, Able-Bodied (NDAB). Inclusion and exclusion criteria, survey administration (SF-36, AUASI, FISI, PAC-SYM, FSFI), pelvic function diaries, stimulation order randomization, electrode and VPP sensor placement, and threshold determination were performed as in our previous study[109]. The primary difference in this study is the alteration of the stimulation and VPP recording protocol (Figure 9) and the addition of erotic stimuli in the form of erotic videos.

Participants were given the choice of a video sequence that contained pornographic scenes between two women or between a man and a woman. The video sequence contains four segments: a 5-minute nature video (baseline or B), a 10-minute erotic video as erotic stimuli (ES-1), a 10-minute continuation of the nature video (recovery or R) during which stimulation is turned on, and a different 10-minute erotic video (ES-2). Between each video segment, there was a 30-second pause for the participant to record their level of subjective arousal on a scale of 1 to 5. To provide privacy, headphones were offered, and vitals (heart rate and blood pressure) were monitored remotely. At the end of each visit, participants were asked if they had a preference between the erotic videos.

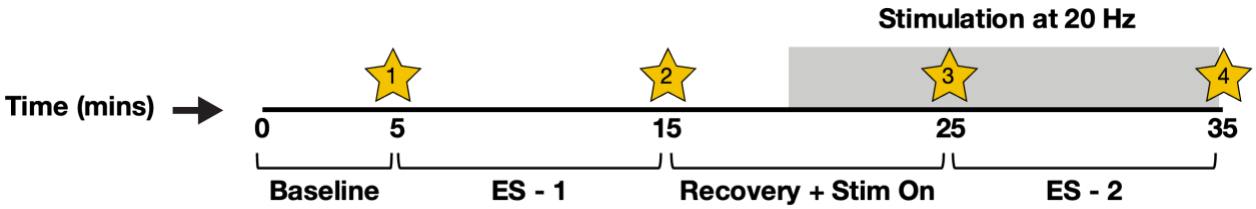

Figure 9. Study recording protocol for GNS and TNS trials. The stars represent the time points at which participants rate their level of subjective arousal.

We processed VPA signals with a bandpass from 0.5 to 5 Hz and calculated peak-to-peak amplitude and binned the average peak-to-peak values in 10-second intervals, as in Chapter 3. Then, we segmented the data for each video, removing 30 seconds of data at the beginning and end of each video segment. This was to exclude artifacts due to potential participant movement while recording their subjective arousal rating. Although the low-pass frequency we chose is lower than the commonly used 30 Hz, VPA is reported in units of relative change (e.g., percent

change or change in mV) and the overall features of the waveform remain intact. We made comparisons between video segments (B to ES-1, B to ES-2, and ES-1 to ES-2) within each participant with a one-way Analysis of variance (ANOVA) followed by post-hoc pairwise Tukey HSD tests. We compared the percent increase in average VPA across participants for each stimulation location and each video (stim on and stim off) with a paired t-test. Comparisons between all statistical analyses used alpha = 0.05 to determine significance.

4.4 Results

Recruitment for this study is ongoing and at the time of this dissertation's publication, a total of 11 participants have been enrolled in the study and 7 have completed both GNS and TNS study visits. Target enrollment for the study is 5, 3, and 5 participants in the NDAB, SCAI, and FSD categories respectively for a total of N = 13. Demographics for each cohort can be found in Table 7.

Table 7. Participant demographic information

Participant ID	Age	Height (m)	Weight (kg)	Race	Ethnicity
NDAB-1	28	1.70	57	White, Caucasian	Non- Hispanic or Latino
NDAB-2	27	1.78	91	Black or African American	Non- Hispanic or Latino
NDAB-3	37	1.63	59	White, Caucasian	Non- Hispanic or Latino
NDAB-4	52	1.60	85	White, Caucasian	Non- Hispanic or Latino
NDAB-5	45	1.73	68	Black or African American	Non- Hispanic or Latino
SCI-1 (C8, AIS-B, 10 years post-injury)	52	1.68	68	White, Caucasian	Non- Hispanic or Latino
SCI-2 (T5, AIS-A, 23 months post-injury)	49	1.60	60	White, Caucasian	Non- Hispanic or Latino
SCI-3 (C6, AIS-D, 20 months post-injury)	48	1.55	63	White, Caucasian	Non- Hispanic or Latino
FSD-1	27	1.60	50	Asian	Non- Hispanic or Latino
FSD-2	42	1.60	59	Asian	Non- Hispanic or Latino
FSD-3	23	1.70	57	Asian	Non- Hispanic or Latino

Participant scores for pre-study pelvic function surveys across cohorts can be found in Table 8. The SCI group and FSD group reported significantly lower FSFI lubrication sub-scores than their NDAB counterparts. The overall FSFI score was also significantly lower in FSD participants and it is worth noting that the average SCI FSFI score was well below the clinical

cut-off score of 26.55[45]. The SCI group also reported a lower FISI score than the NDAB group. All other survey scores were not significantly different between participant groups.

Table 8. Participant pre-study survey results

Participant ID	SF36 (0 to 100^{+})	AUASI (35 to 0^{+})	PACSYM (48 to 0^{+})	FISI (61 to 0^{+})	FSFI (2 to 36^{+})	FSFI Lubrication (1 to 6^{+})
NDAB-1	83	1	4	6	31.4	5.7
NDAB-2	66	11	4	7	23.9	5.4
NDAB-3	66	4	5	0	23.9	4.8
NDAB-4	89	3	3	0	27.8	5.7
NDAB-5	93	8	0	0	26.3	5.4
SCI-1	70	0	6	8	22.9	2.1
SCI-2	63	1	3	22	4.4	0
SCI-3	66	13	4	23	20.3	3.3
FSD-1	71	3	4	4	12.1	3.6
FSD-2	85	3	27	20	14.3	2.7
FSD-3	94	9	3	0	18.8	4.8
Average NDAB	79.4	5.4	3.2	2.6	26.7	5.4
Average SCI	66.4	4.7	4.3	17.7*	15.9	1.8*
Average FSD	83.3	5.0	11.3	8.0	15.1*	3.7*

- * $p < 0.05$ compared to NDAB group with student's t-test
- + indicates "ideal health" survey score

In GNS trials we found that in 5 of the 8 trials, there were significant increases in the VPA responses from baseline to the first erotic video (B to ES-1 in Figure 10). In the 3 other trials without significance, participants were in the SCI or FSD group. In all GNS trials there was a significant difference between baseline to the second erotic video, when stimulation is turned

on (B to ES-2 in Figure 10). Across participant groups, the SCI group had a significantly lower increase in VPA than the NDAB group in response to ES-1. All other comparisons (B to ES-1, B to ES-2, and ES-1 to ES-2) were not significant. Some individual participants (SCI-1, SCI-2, FSD-3) had relatively low VPA responses compared to other study participants. SCI-2 and FSD-3 had a negative VPA response during ES-1 in the GNS session. Five out of 8 TNS trials had a higher VPA response for ES-2 than ES-1.

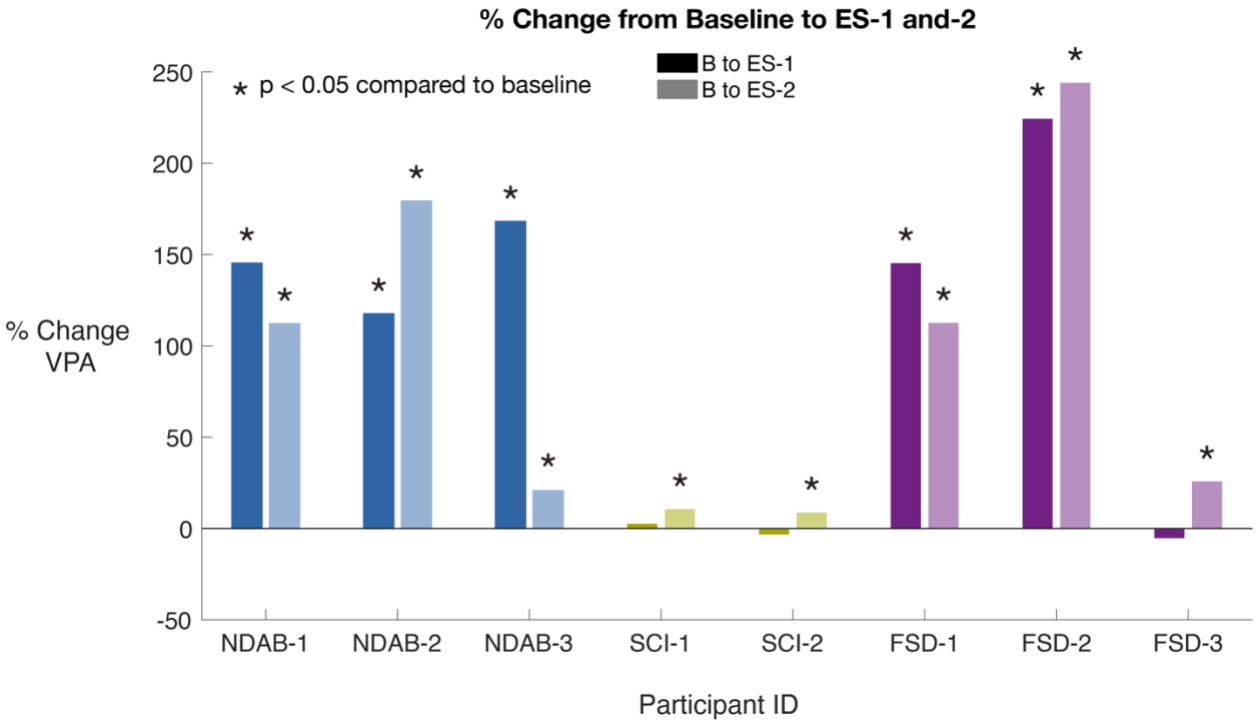

Figure 10. Bar chart of the percent increase in vaginal pulse amplitude (VPA) from Baseline to ES-1 and from Baseline to ES-2 during genital nerve stimulation trials. Blue, green, and purple indicate NDAB, SCI, or FSD cohort. * denotes significance difference from baseline ($p < 0.05$).

In TNS trials we found all individual VPA responses during ES were significantly different from baseline (Figure 11). Across participants, the FSD group had a significantly higher VPA response from B to ES-1 and B to ES-2. All other comparisons across VPA responses between participant groups were not significant. One participant in the NDAB cohort had a decrease in VPA during both ES videos. Seven out of ten TNS trials had a higher VPA response for ES-2 than ES-1.

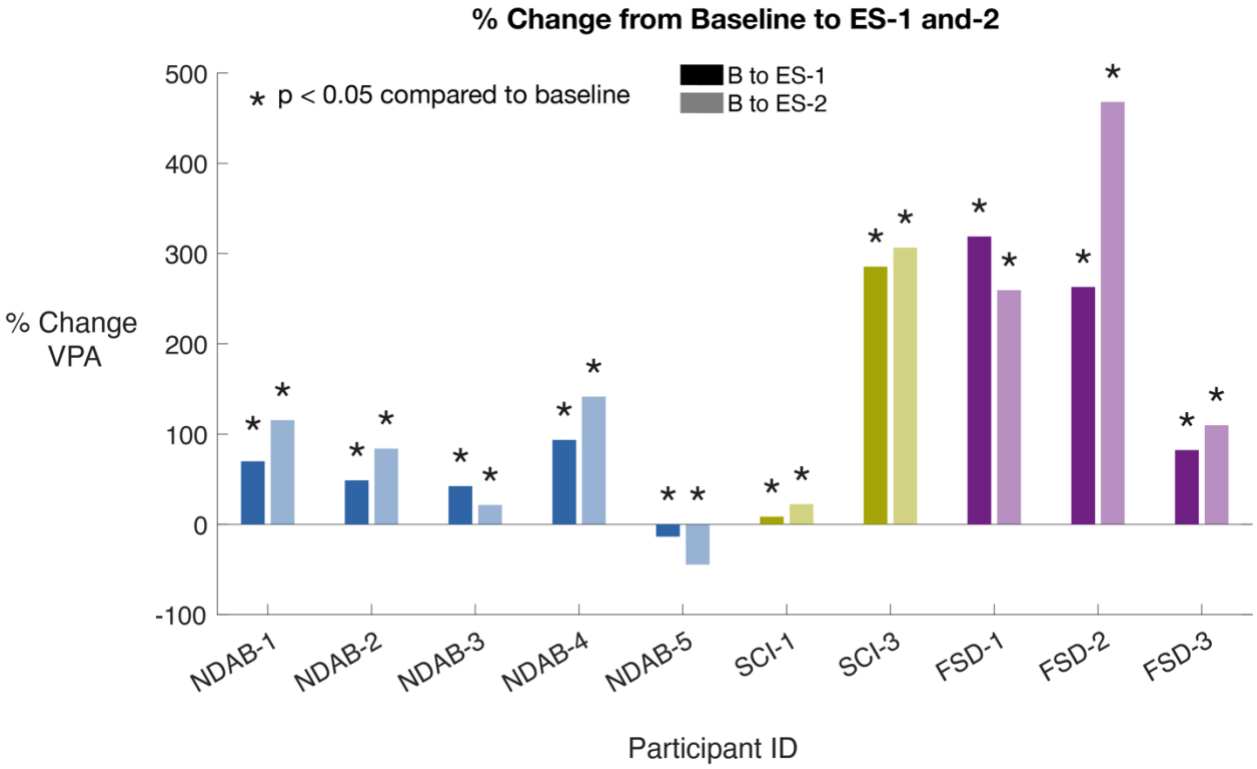

Figure 11. Bar chart of the percent increase in vaginal pulse amplitude (VPA) from Baseline to ES-1 and from Baseline to ES-2 during tibial nerve stimulation trials. Colors correspond as in Figure 10. * denotes significance difference from baseline ($p < 0.05$).

In comparing ES-1 and ES-2, we found a wide range of % difference across all trials, roughly -60 to 60%. In Figure 12, a positive percentage represents a higher VPA response

during ES-2, when stimulation is on. Conversely, negative values indicate that ES-1 had a higher VPA response than ES-2. There were no statistical differences between the VPA response of ES-1 and ES-2 across participant groups. Stars in Figure 12 indicate that when prompted at the end of the session, the participant indicated they enjoyed the video which provoked a higher VPA response more, indicating some level of sexual concordance between genital and subjective sexual preferences.

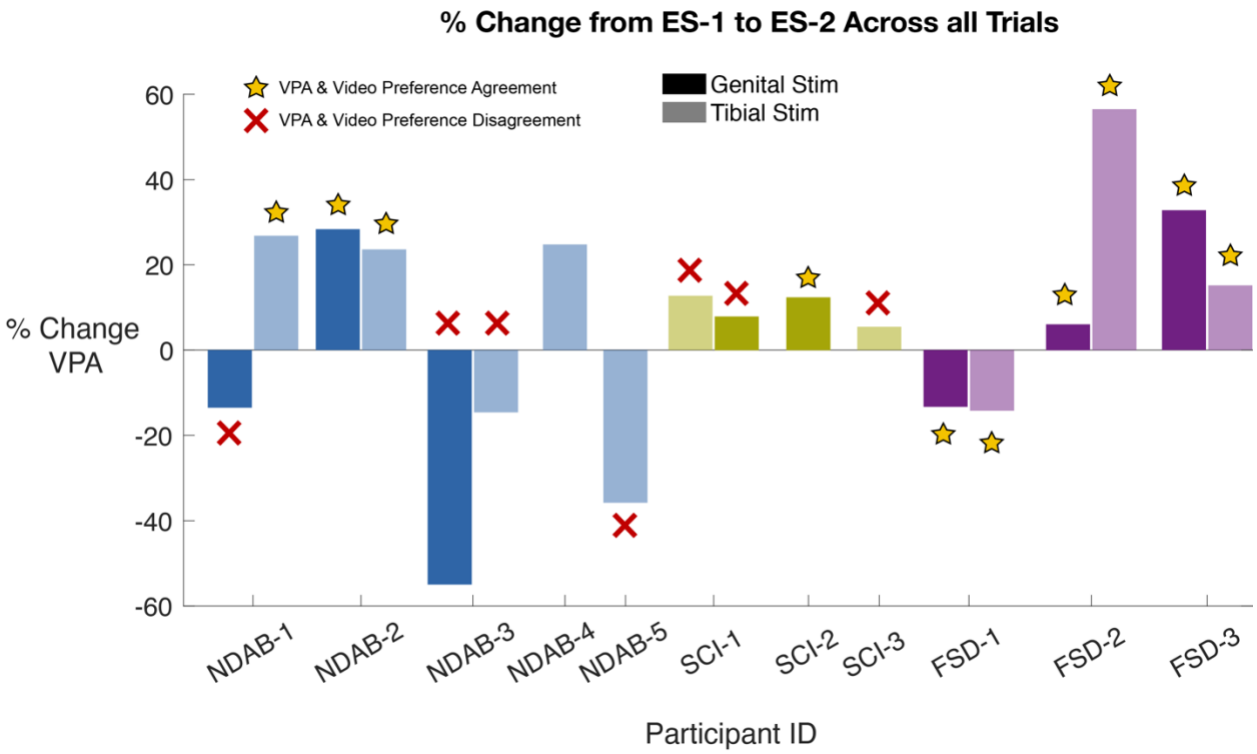

Figure 12. Bar chart of the percent increase in vaginal pulse amplitude (VPA) from ES-1 to ES-2 across all trials. Colors correspond as in Figure 10. A star indicates that the participant preferred the erotic video corresponding with a higher VPA response. An X indicates the inverse. NDAB-4 had no video preference.

4.5 Discussion

Our goals for this study were to investigate the effect of a single session of transcutaneous GNS or TNS on genital arousal. Building upon the previous study, we incorporated erotic stimuli to align with established paradigms in sexual function research. A majority of participants (5/8 and 7/10 in GNS and TNS, respectively; 67% overall) had higher VPA responses to ES-2, the erotic video segment with stimulation (Figures 10,11). Additionally, we found that only the FSD cohort had sexual concordance across all trials (Figure 12).

There were unexpectedly high VPA responses in the FSD cohort relative to the NDAB cohort both with and without stimulation (Figure 10, Figure 11). We screened FSD participants by requiring an FSFI lubrication sub-score less than 3. Knowing that genital blood flow is a critical step in the production of lubrication, we did not expect any FSD VPA responses to be significantly higher than NDAB. Perhaps non-neurogenic FSD increases sensitivity to visual erotic stimuli, leading to a higher VPA response. To further investigate the correlation or concordance between genital arousal (VPA response) and subjective arousal (participant experience), a larger sample size and more targeted questions about the participant's experiences during the study session are warranted.

The highest VPA responses across all participants were observed during TNS sessions, and seven out of ten TNS sessions had higher VPA responses with stimulation than without (Figure 11). However, there are two considerations to keep in mind when interpreting these

results. Firstly, the stimulation period is always during ES-2 in the video sequence, and it is possible that it was not enough time for the VPA signal to return to baseline. This is a common assumption made in erotic video study paradigms but is not always accurate. Secondly, most participants preferred the second video (Figure 12) so it is possible that their increased engagement with the video, rather than a response to the stimulation, caused a higher VPA response.

In five out of eight GNS trials, the ES-2 VPA response was higher than ES-1 (Figure 10). These results are mixed across participants, which is unsurprising given the limited sample size. The same two considerations (stimulation order and video preference) apply to GNS VPA response results as for TNS VPA responses.

This work supports the potential of TNS and GNS as a therapy for FSD as participants indicated their willingness to try it and provided positive feedback about both modalities. However, to establish more conclusive evidence, more rigorously controlled studies with larger sample sizes are necessary. This research provides pilot data that can guide further investigations into TNS and GNS as a therapy for FSD, such as a longitudinal study that measures VPA responses over time with repeated stimulation sessions as an intervention.

4.6 Conclusion

In conclusion, our study aimed to investigate the effects of short-term transcutaneous GNS and TNS on genital arousal and sexual concordance. We observed higher VPA responses

during the stimulation period in 5 of 8 GNS trials and 7 of 10 GNS trials. Unexpectedly, participants with FSD exhibited higher VPA responses than the NDAB cohort. Understanding which factors contribute to sexual concordance and finding a biomarker with high sexual concordance is imperative to guide future research in this area.

Chapter 5 Discussion

In this dissertation, I investigated the effect of genital and tibial neuromodulation on genital hemodynamics in translational spinal cord injury studies. I hypothesized that genital and tibial nerve stimulation may improve female sexual dysfunction symptoms by improving genital arousal. The research in this dissertation is the first to study vulvar hemodynamics in animals and the effect of neuromodulation on genital blood flow in humans. Although physiological results were not consistent across studies, women with spinal cord injury and women with sexual dysfunction indicated their willingness to use transcutaneous neuromodulation as a treatment for sexual dysfunction. This dissertation work provides further support for genital and tibial neuromodulation as a treatment for female sexual dysfunction, preliminary data for future, more rigorous studies, and highlights the need for better biomarkers in the field of female sexual medicine.

In Chapter 2, I investigated the effect of pudendal nerve (distal branch of the genital nerve) and tibial nerve stimulation on perineal blood flow before and after a thoracic level spinal cord transection in anesthetized female rats. This was the first study to measure vulvar blood flow in an animal model. We found that pudendal, but not tibial, nerve stimulation led to significant increases in vulvar blood perfusion while stimulation was on. We hypothesize this was caused by efferent motor activation that contracted pelvic floor musculature, increasing blood flow demand to the pelvic region.

These results were unexpected as we hypothesized that both genital and tibial nerve stimulation would lead to significant increase in vulvar blood perfusion that persisted after stimulation was turned off. This hypothesis was based off preclinical rodent studies that found pudendal and tibial nerve stimulation led to increases in vaginal blood perfusion[34,68,71]. The distinction between vaginal and vulvar blood flow is an important one and researchers are finding higher sexual concordance between vulvar blood perfusion and subjective arousal[46], indicating that vulvar blood perfusion may be a better biomarker for genital arousal than vaginal blood flow. An improved animal model that incorporates simultaneous measurements of vaginal and vulvar blood perfusion may provide further insights into the neurophysiology of genital arousal. Additionally, it would be interesting to incorporate brain activity measurements of the thalamus, MPOA, or other brain regions known to be involved in sexual function as discussed in Chapter 1. This would increase the robustness of a spinal transection rodent model by 1) identifying shared brain structures between humans and an anesthetized rodent model and 2) providing a negative control for post-transection brain measurements. Another animal model could use a spinal cord contusion model aimed at different tracts of the spinal cord. With this set-up, one could perform tibial nerve stimulation and confirm it increases vaginal blood flow, deliver a contusion to an area known to be involved relaying genital information such as the spinothalamic tract, and then measure the response again to see if the response is dampened. Afterwards, the spinal cord region that was damaged could be confirmed with staining or other imaging techniques.

In Chapter 3, we investigated the effects of one-time transcutaneous genital nerve stimulation and tibial nerve stimulation on genital and subjective arousal in women with FSD, women with SCI, and healthy controls. To our knowledge, this is first study to measure genital arousal (using vaginal photoplethysmography) in response to peripheral neuromodulation. We found a significant increase in subjective arousal in GNS, but not TNS, trials. The effect of stimulation on vaginal blood flow varied across stimulation sessions and participant groups. We hypothesize that this was primarily due to the descending pathways from the brainstem, inhibiting genital arousal due to the inappropriate setting. Perhaps a study with a larger sample size would reveal trends between VPA response and participant group. Notably, all participants with complete SCI experienced genital sensations during at least one study visit, despite not typically have any feeling. We hypothesize this sensory information is being carried via vagus nerve afferents[151] or residual fibers in the spinal cord[62]. The absence of sexual stimuli in the study protocol limited direct comparisons with other studies that measure VPA, as is common in the field of sexual medicine[137]. However, this study provided preliminary data for VPA signals in the absence of visual stimuli which builds a foundation for further investigating the variability of VPA signals and their utility as a sexual function biomarker.

In Chapter 4, we added a fixed sequence of neutral and erotic videos to the study protocol and recruited participants in the same cohorts as in Chapter 3. We made comparisons within and across participants genital and subjective arousal between 3 video segments: Baseline, ES-1, and ES-2. Almost all participants had significant increases in vaginal blood flow from Baseline to

ES-1 and ES-2. We also found that most participants had higher VPA responses (% change from baseline) during the second erotic video, which was also the stimulation period during GNSand TNS trials. The majority of participants also indicated they preferred the second erotic video. However, it is unclear how much of a role TNS or GNS played in a high VPA response to ES-2, given the confounding factors of video preference and video order. A future study could improve upon this work by incorporating blinding stimulation periods for participants as well as randomized stimulation and video periods. Another study may want to look at longitudinal changes over time, to better parallel studies that found improvements in survey reported FSD in response to repeated PTNS[66].

Sexual concordance varied across NDAB and SCI groups, but all participants in the FSD cohort had sexual concordance in that their preferred video was the one in which they had a higher VPA response (ES-1 to ES-2). This was not entirely unexpected as the importance of sexual concordance is not yet fully understood[9]. FSD participants' high VPA response in Chapter 4 as well as their low subjective arousal score in Chapter 3 were both unexpected, which may indicate the field of sexual medicine needs to continue investigating the utility use of the FSFI in categorizing women with FSD. A future study could improve upon this work by recruiting more women with FSD and conducting a more detailed assessment of their specific dysfunction or specifically recruiting women with female sexual arousal/interest disorder or genitourinary symptom of menopause, both of which we know impacts the physiology of arousal. This would help categorize the different types of FSD, so that we may better understand their different

etiologies and provide more targeted treatment options. Additionally, I would recommend that studies keep track of participants menstrual cycle as well as any medications that alter their hormone levels as hormones like estrogen and testosterone can be used as treatments for sexual dysfunction.

The next steps for this line of research need to focus on understanding the differences between genital and subjective arousal. Unfortunately, subjective arousal is hard to ascertain in both animal and human studies. For animals, they must be awake and behaving which presents a large and intricate data collection problem. For humans, everyone's life experiences heavily influence their subjective experiences, and thus, their subjective arousal when presented with different sexual stimuli. To ensure subjective arousal has maximum potential, I would recommend that any clinical study allows participants to bring their own pornographic material. A future clinical study may want to measure genital arousal during a 5-minute neutral baseline, the participants chosen material, and a recovery period until baseline levels are re-established. This would introduce recovery time as an additional metric for evaluating sexual dysfunction and accommodate a wider array of subjective arousal responses.

In conclusion, I found that genital and tibial neuromodulation may be viable treatment options to improve genital arousal in people with FSD and that women with SCI and FSD are receptive to transcutaneous electrical nerve stimulation. This dissertation also lays additional groundwork for a translational approach to quantifying genital arousal responses. It also highlights the need for the field of sexual medicine to improve upon their definitions of sexual

dysfunction and for researchers to identify genital arousal biomarkers that agree with the individual's subjective experience.

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