Pudendal, but not tibial, nerve stimulation modulates vulvar blood perfusion in anesthetized rodents

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Abstract

Introduction: Genital arousal is a necessary component of sexual function but has been understudied in women. There are few treatment options for women who have female sexual dysfunction stemming from difficulties with arousal. Some studies have shown that neuromodulation can increase vaginal blood perfusion, but the effect of neuromodulation on blood perfusion in the vulva is unknown.

Aim: Our goal was to investigate whether pudendal or tibial nerve stimulation can 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 using laser speckle contrast imaging in response to twenty-minute stimulation trials of pudendal and tibial nerve stimulation. Then, after thoracic level spinalization and a rest period, we repeated these trials.

Main Outcome Measures: We calculated average blood perfusion for three perineal regions (vulva, anus, and inner thigh) before, during, and after stimulation. We made comparisons for each region before, during, and after 20 minutes of stimulation and before and after spinalization.

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

Keywords: blood flow, neuromodulation, pudendal nerve, tibial nerve, vulva, laser speckle imaging

Introduction

Female sexual health is an important determinant in quality of life, contributing to both increased meaning in life and general well-being¹. Unfortunately, approximately 40% of women suffer from female sexual dysfunction (FSD)². 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 arousal³. Bremelanotide and flibanserin, both approved to treat hypoactive sexual desire disorder (HSDD), have shown mild efficacy in improving sexual desire and arousal^{4,5}. However, bremelanotide has been associated with a high incidence of adverse events⁶ and a review of flibanserin, suggests that it has minimal clinical benefit⁷. Sildenafil citrate was studied as a treatment for female arousal disorders and demonstrated an ability to increase clitoral and vaginal vasocongestion, both important steps in the sexual response cycle^{8,9}. Unfortunately, sildenafil citrate was not further pursued as a treatment option because its success at increasing genital arousal did not translate into improvements in subjective arousal and it was found to have a high incidence of adverse events^{10,11}.

Neuromodulation is a potential treatment option for women with FSD. Sacral neuromodulation (SNM) is a standard treatment for overactive bladder and fecal incontinence^{12,13}. 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^{14–18}. Similar improvements in sexual function have been seen in clinical studies that used tibial nerve stimulation to treat bladder dysfunctions^{19,20}. 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 dorsal nerve, a distal branch of the pudendal nerve, improved survey-reported FSD symptoms²¹. 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 receptivity²². 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 onset²³. 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 minutes²⁴ or up to 30 minutes²⁵ after stimulation onset depending on stimulation parameters. Both pudendal and tibial nerve stimulation have the potential to improve sexual function, but a greater understanding about the underlying mechanisms and 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 arousal²⁶. Although paraplegic patients report that regaining sexual function after SCI is a priority^{27,28}, women with SCI have the same FSD treatment options as non-neurogenic women. These treatment options do not consider the impact of neurological dysfunction. 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 the physiological response to receptive sexual stimuli and is characterized by an increase in genital blood flow, engorgement of the genitals, and the production of lubrication²⁹. Most research studying female sexual responses use vaginal photoplethysmography to measure genital arousal³⁰. However, researchers in the gynecological fields 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^{31,32}.

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 studies³¹⁻³⁴, this is the first time it has been used to measure perineal blood perfusion in animals. We found that during the stimulation epoch, pudendal, but not tibial, nerve stimulation causes an increase in perineal blood perfusion. There were no differences in perineal blood

perfusion after stimulation was delivered, for either nerve target, when compared with baseline. We found that these response patterns were not altered by thoracic spinal transection and are likely due to direct efferent activation of the pelvic floor musculature.

Methods

Animal Surgery

All experimental procedures were approved by the governing 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)³⁵. We used ten animals for primary experimental testing, as described below. We selected this sample size based on prior animal studies with similar objectives^{25,36,37}. 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^{24,38–40}. 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 stage⁴¹.

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 μ m 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. We

then closed the incision and moved the animal to a supine position. We then placed a percutaneous electromyogram (EMG) wire (stainless steel, 50 μ m, 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 1A).

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 rat, tibial nerve stimulation was performed first. We then semi-randomized the order of the stimulated nerve across following animals 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.

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 1B). 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.

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 online⁴².

Intact Spinal Cord Recordings

During pudendal nerve stimulation trials (Figure 2A), the average VBP at baseline was 184 ± 44 APU, which increased to 331 ± 129 APU during stimulation ($+80.9 \pm 64.2\%$ 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 ($+97.6 \pm 74.4\%$), and 169 ± 44 APU after stimulation ended. The average ITBP was 94 ± 33 , 154 ± 67 ($+67.0 \pm 63.7\%$), 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 ($p < 0.01$).

The average VBP for tibial nerve stimulation trials (Figure 2C) was 183 ± 50 APU prior to stimulation, 192 ± 48 APU during stimulation ($+5.7 \pm 11.9\%$), and 181 ± 52 APU after stimulation ended (Figure 3C). For the same trials, ABP was 163 ± 42 , 163 ± 42 ($+0.4 \pm 8.0\%$), and 162 ± 47 APU before, during, and after stimulation. ITBP had mean values for the three periods of 85 ± 26 , 93 ± 34 ($+7.8 \pm 22.5\%$), 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.

Post-Spinalization Recordings

Pudendal nerve stimulation trials after spinalization (Figure 2B) had an average VBP of 193 ± 61 , 337 ± 131 ($+74.5 \pm 42.8\%$ 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 ($+86.6 \pm 59.1\%$), and

167 ± 37 APU. Average ITBP was 88 ± 30, 153 ± 63 (+74.4 ± 60.7%), and 86 ± 28 APU (Figure 3B).

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 2D). 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 3D).

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 4B). 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 4A). 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.

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).

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 3). Tibial nerve stimulation in intact animals did not impact blood flow during or after stimulation was delivered (Figure 2C). After spinal cord transection, pudendal and tibial nerve stimulation had the same effect as during intact spinal cord trials (Figure 2C/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 onset⁴³. This muscle activation causes a generalized increase of blood flow to the entire perineal region. Spinal cord transection (Figure 3B) had no impact on the pudendal stimulation response while transecting the pudendal nerve distally abolished the response, indicating that the neural pathways involved are likely direct efferents. 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^{24,25}. 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 lasting for 15 seconds to two minutes. Preclinical experiments have also demonstrated the potential for tibial nerve stimulation to modulate vaginal blood flow^{23,44} and that estrogen is necessary to preserve this response⁴⁴. We expected to see similar responses in perineal blood perfusion in our experiments, but 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 arteries⁴⁵. In contrast, the perineal region, which includes the vulva and anus, is only supplied by the internal pudendal arteries⁴⁶. 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)³⁰. However, more researchers are using non-invasive techniques such as Laser Doppler Imaging (LDI)^{31,32,34} and LSCI³³ 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. Perhaps nerve stimulation is not 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 2, 3) is not entirely unexpected, as the tibial nerve does not directly innervate the perineal region. 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^{23,44}. 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^{47,48} populations in weekly percutaneous stimulation sessions. 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. Perhaps

different stimulation parameters or repeated stimulation sessions (as is common for clinical treatment) would 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 3). 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 artery⁴⁹. 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 and not a region-specific response.

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 dorsal 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 function²¹. 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 inconsistency with prior preclinical studies is due to a difference in blood perfusion location. 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 how vaginal and vulvar blood perfusion relate to one another.

Conclusions

This is the first preclinical study to measure female rat perineal blood perfusion using LSCI in response to nerve stimulation. We found that vulvar and anal blood perfusion increased during pudendal,

but not tibial, nerve stimulation. Spinalization had no impact on the perineal blood perfusion response to either stimulation target. Further research is needed about the concordance between vaginal blood perfusion, vulvar blood perfusion, subjective arousal, and lubrication is necessary before using vulvar blood perfusion as a measurement of genital arousal in anesthetized animal models.

Disclosure

The authors report no conflicts of interest in this work.

Table 1. Summary of experiment parameters

Animal ID	Weight (kg)	First Nerve Target	Estrous Stage	Intact Spinal Cord		Spinalized	
				Pudendal Nerve MT (μ A)	Tibial Nerve MT (μ A)	Pudendal Nerve MT (μ A)	Tibial Nerve MT (μ A)
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

Figures

Figure 1. Experimental set-up. Figure 1A shows a diagram of the LSCI with the laser aimed at the vaginal vestibule of a rat lying in the supine position. Figure 1B shows an example of a laser speckle image 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.

Figure 2. Individual (thin, colored lines) and average (solid black line) blood perfusion responses for all animals that completed the entire nerve stimulation protocol (n=10). Top left panel shows pre-spinalization pudendal stimulation trials. Blood perfusion returned to baseline after stimulation was turned off at 25 minutes. The top right panel shows pudendal stimulation trials after spinalization and follows similar trends as pre-spinalization trials. The bottom panels depict tibial nerve stimulation trials for intact (left) and spinalized (right) trials.

Figure 3. 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.

Figure 4. 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).

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