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The effect of spinal cord injury on sexual function.

Casey J. Steadman
University of Louisville

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By

Casey J. Steadman
B.S., Mississippi State University, 2012
M.S., Mississippi State University, 2014
M.S., University of Louisville, 2017

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A Dissertation Approved on

June 17, 2019

By the following Dissertation Committee:

_____
Dissertation Director
Dr. Charles H. Hubscher

_____
Dr. Susan J. Harkema

_____
Dr. Ashok Kumar

_____
Dr. Alexander V. Ovechkin

_____
Dr. David S. Magnuson

_____
Dr. Jeffrey C. Petruska

DEDICATION

This dissertation is dedicated to the people who make my heart whole

My husband, Charlie

and

My daughters, Clementine and Juniper

for their unwavering love, understanding, and support.

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ABSTRACT

THE EFFECT OF SPINAL CORD INJURY ON SEXUAL FUNCITON

Casey J. Steadman

June 17, 2019

Spinal cord injury (SCI) causes severe neurological impairment with widespread motor, autonomic and sensory deficits, leading to a substantial quality of life impairment. The number of individuals with SCI increases by approximately 12,500 annually, and over 80% of individuals with SCI are males. SCI individuals rate sexual function as a top priority quality of life issue, and men with SCI likely suffer from sexual dysfunction, such as erectile and ejaculatory dysfunction, as well as infertility. Regardless of the high status of importance of sexual function, limited numbers of experimental studies in SCI animal models have focused on sexual function after SCI. Interestingly, human clinical research participants with SCI undergoing daily locomotor treadmill training have reported changes in sexual function. To advance targeted recovery of sexual function after SCI, a better understanding of the post-injury neural circuitry is necessary.

The objective of this project was to determine the effect of activity-based training (ABT) on sexual function in a rat model of SCI and examine the effects of a contusion SCI on sexual function using improved quantifiable measures. Two well-established measures of sexual function were used to determine the effects of ABT on sexual function after SCI: the penile dorsiflexion reflex (PDFR) and

bulbospongiosus electromyography. The second and third studies focused on determining the effect of a clinically relevant contusion injury of varying severities using kinematic analysis of the PDFR and real-time intracavernosal pressure recordings from the penis of rats undergoing mating behavior testing.

ABT was shown to positively affect sexual function after SCI, where task-specific stepping and/or hindlimb loading impacts the local spinal cord circuitry responsible for sexual function. ABT may strengthen the residual fibers crossing the lesion epicenter, providing better coordination of supraspinal influences on the spinal cord sexual circuitry. Sexual function after SCI was determined to be correlated with the percent of white matter spared (a measure of injury severity), further supporting the disruption of spinobulbospinal coordination as the cause of sexual dysfunction after SCI. Kinematic analysis of the penile dorsiflexion reflex and telemetric recording of intracavernosal pressure may provide improved quantifiable measures for examining sexual function after SCI.

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CHAPTER I

GENERAL INTRODUCTION

Sexual function after spinal cord injury: innervation, assessment, and treatment

Over 1.2 million Americans live with paralysis from spinal cord injury (SCI) with approximately 12,500 new cases reported annually. The majority of SCI individuals are males (80.7 %) who are twice as likely to incur a SCI compared to females[1]. Among SCI individuals, sexual function is rated a top priority due to the impact on quality of life (QoL) [2, 3]. High scoring on either the Sexual Interest and Satisfaction Scale (SIS) or the Sexual Adjustment Scale (SAS) has been shown to correlate to higher QoL scores using a visual analogue scale[4, 5]. About 61 % of both male and female SCI individuals rate their sexual life as satisfactory, with men being less satisfied than women [6, 7]. Despite the high priority, only a limited number of experimental animal studies have focused on sexual dysfunction post-SCI[3, 8].

Males

Erectile function. Spinal shock immediately post-SCI severely depresses or eliminates male genital reflex function. Spinal shock may last from several hours to several weeks after injury, making predictions of sexual recovery difficult[9]. Once

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the spinal shock period has ended, reflexogenic, psychogenic, and mixed erections (both reflexogenic and psychogenic in origin) can be induced[10, 11]. Erection stems from the activation of the autonomic nervous system, with parasympathetic input being pro-erectile and somatic input assisting with erectile function. Parasympathetic outflow originates in the sacral parasympathetic nucleus (SPN) of the S2-S4 spinal cord in humans (L6-S1 in rodents) and reaches the penis via the pelvic plexus and cavernous nerves[12-15]. Parasympathetic excitatory input to the penis via these nerves is responsible for vasodilation of penile vasculature, increased penile volume, and increased intracavernous pressure[15-20].

The 2014 NSCISC database indicates that most SCI lesions are above the sacral level[1, 21]. Sensory input from the penile skin, glans, and prepuce project to pudendal motoneurons of Onuf's nucleus within the sacral cord (human; dorsomedial and dorsolateral nuclei in the rat) to cause contractions of the bulbospongiosus (BS) and ischiocavernosus (IC) muscles, which aids in rigidity and maintenance of erection[12, 15, 22, 23]. Loss of descending inhibition from the brain after SCI allows for reflexogenic erections in response to cutaneous stimulation of the genital region as sacral circuitries remain intact. However, these reflex types of erections are often short-lived and lack sufficient rigidity for penetration[11, 24]. Studies in the rat indicate that lesions of several different supraspinal sites, including the median and pontine raphe nuclei, lateral paragigantocellular nucleus, and nucleus of the medial amygdala, significantly increase the occurrence of reflexogenic erections, suggesting an inhibitory role and potential sources of the damaged projections (either direct or indirect) after SCI[25-27]. In human studies

using positron emission tomography to measure regional cerebral blood flow, deactivation of the amygdala and ventral temporal lobe has been shown to coincide with genital stimulation, which is hypothesized to be associated with decreased vigilance during sexual performance[28].

Psychogenic erections require an intact thoracolumbar sympathetic pathway for activation via visual and auditory stimuli, along with memories and fantasies[10, 11]. The capacity for psychogenic erections post-injury is likely low, as only 5.9 % of SCI individuals have lesions below L2[1]. Sympathetic innervation of the penis arises from two distinct spinal areas: the intermediolateral cell column (T12-L2) and the dorsal grey commissure (L1-L2). The intermediolateral cell column contains neurons that reach the penis via the lumbar paravertebral sympathetic chain and the hypogastric nerve, while the dorsal grey commissure sends axons via the hypogastric nerve alone[13, 29]. The role of the hypogastric nerve in penile erection has been a topic of debate. Depending upon the author and animal model studied, the hypogastric nerve may have anti-erectile effects, pro-erectile effects, or no effect at all, suggesting the possibility of efferent fibers with differing roles[18, 30, 31]. Psychogenic erections persist when descending pathways from brain nuclei projecting directly to the sacral cord remain intact, suggesting a common white matter location of sympathetic and parasympathetic descending axons. Supraspinal regions that have been implicated in animal models for controlling non-contact erections include the hypothalamic paraventricular nucleus, bed nucleus of the stria terminalis, and medial nucleus of the amygdala[27, 32-34]. Additionally, the ventral

putamen and the hypothalamus have been suggested to aid in maintenance of psychogenic erection[35].

Ejaculation. In addition to erectile dysfunction, SCI males have adversely affected ejaculatory function. Anejaculation affects a significant portion of SCI males[8, 10]. Approximately 95 % of SCI men with lesions caudal to T10 have severe impairment of ejaculation, with ejaculation occurring during self-stimulation or coitus at very low rates[36-38]. Fertility is also negatively affected due to necrospermia (non-viable sperm with low motility), hypogonadism, and hypospermatogenesis[39-42].

Ejaculation consists of two successive events: emission and expulsion[43, 44]. Anejaculation in SCI males may be due to a lack of emission of seminal fluid into the urethra[45]. During the emission phase of a healthy male, ejaculate fluid enters the posterior urethra as sensory penile afferents signal the emission center of the T10-L3 spinal cord[46]. Efferent autonomic signals dominated by sympathetic nerves of the hypogastric plexus then cause contractions of the vas deferens, prostate, bladder neck, and seminal vesicles. Seminal fluid is released into the posterior urethra, with the contracted bladder neck preventing the fluid from retrogradely entering the bladder[47, 48]. However, impairment of bladder neck musculature control in SCI may lead to retrograde ejaculation of seminal fluid into the bladder[49, 50].

During expulsion, seminal fluid is released from the glans meatus[51]. Impulses within somatic efferents from Onuf’s nucleus and parasympathetic efferents from the SPN of the S2-S4 spinal cord propagate via the pudendal and

pelvic nerve, respectively, to cause pulsatile contraction of the urethralis, BS, and IC muscles. Rhythmic contraction of these muscles forces seminal fluid to exit the urethra to the exterior[48, 52, 53]. In SCI men where this somatic-parasympathetic coordination is impaired but ejaculation is possible, the ejaculate is not forcefully expelled but dribbles out the urethra[54].

Preclinical models that have mapped pathways mediating the control of ejaculation include supraspinal projections from regions such as the medial preoptic area, the paraventricular nucleus, the lateral hypothalamus, and the lateral paragigantocellular nucleus[55-58]. Descending projections include the medullary reticular formation (MRF) whose nuclei have been shown to be critical in male ejaculation, along with both receiving and sending projections to other brainstem nuclei that receive pelvic visceral input, such as the gracile and solitary nuclei[59-68]. Human imaging studies using positron emission tomography indicate that the mesodiencephalic junction, striatum, cerebral cortex, and cerebellum are activated during ejaculation[69]. Despite the loss of supraspinal connections in many SCI males, ejaculatory reflexes have been activated in the SCI male via electro ejaculation (EEJ) and penile vibratory stimulation (PVS) suggesting a spinal generator for ejaculation[43, 70, 71]. Support for such a generator comes from pre-clinical studies by Truitt and Coolen who identified a population of lumbar spinothalamic cells located in laminae VII and X in the L3-L4 rat spinal cord which, when inactivated, cause disruption of the ejaculatory process. Additionally, these cells were shown to have projections to neurons of the intermediolateral cell column and the SPN as well as ascending projections to the parvocellular subparafascicular

nucleus of the posterior thalamus[72]. As ejaculation requires coordination between somatic, sympathetic, and parasympathetic input and output, the location and projections of lumbar spinothalamic cells implicate them as major components of the spinal ejaculation generator.

Females

Sexual arousal. Sexual function is also rated a top priority for females living with paralysis. Like males, after spinal shock subsides, SCI women can experience sexual arousal via reflexive and psychogenic pathways[42, 73-75]. Reflexive arousal has been shown in women with upper motor neuron damage affecting supra-sacral segments and lower motor neuron incomplete injuries[76]. Parasympathetic fibers originating from the S2S4 spinal cord are responsible for the female sexual arousal reflex, which consists of vulvar swelling, vaginal lubrication, tenting, vasocongestion, and clitoral erection[77, 78]. In reaction to genital stimulation, pudendal afferents innervating the clitoris, perineum, and urethra participate in a polysynaptic reflex with the cavernous nerve to lengthen and increase blood flow to the vagina and increase blood flow and intracavernosal pressure in the clitoris, allowing for clitoral erection[78, 79]. Additionally, pudendal sensory fibers activate pudendal motor neurons that contract the striated perineal muscles, allowing for persistence of clitoral erection[23]. During vaginal tenting, the cervix, innervated by both the hypogastric (sympathetic) and pelvic (sensory) nerves, retracts and softens[77, 80, 81]. At this stage, the uterus (innervated by hypogastric and pelvic nerves) moves upward[81-83]. The movement of the cervix and uterus are believed to be initiated

by pelvic muscle contraction and maintained by the smooth muscle contractions of the parametrial sheaths attached to the uterus[82, 84, 85]. Psychogenic arousal via audiovisual cues has been shown to occur in women with intact sensory perception of the T11-L2 dermatomes, and evidence for sympathetic elucidation of psychogenic genital arousal exists in SCI females[74, 86]. Both reflexive and psychogenic arousal is seen in SCI women with incomplete injuries; however, it has been suggested that neither reflexive nor psychogenic arousal is seen for a lesion between T10-T12[73, 75, 87, 88].

Orgasm. The female orgasm consists of rhythmic contractions of the striated perineal muscles controlled via motor pathways of the pudendal nerve, including the BS and IC muscles, and external anal and urethral sphincters[77]. Though arousal is dependent on the level of injury, Sipski et al. reported no statistically significant differences in the ability to reach orgasm in the laboratory setting when comparing between lesion levels, completeness of injury, or upper or lower motor neuron injury. In one study, 55 % of SCI women reported being able to achieve orgasm post-injury though latency to reach climax was significantly longer than able-bodied females[87]. These data suggest that neurological ability to orgasm remains intact; however, psychological and/or extrinsic variables, such as medication or misinformation concerning sexual ability, may inhibit an individual from reaching climax[88].

Menstrual cycles, if affected, return to normal within a year of injury for 90 % of individuals[89, 90]. Thus, SCI women are able to conceive and carry to full term

with no increases in rates of spontaneous abortion relative to the general population[89, 91]. Close observation by an obstetrician is necessary due to increased complications from urinary tract infections (UTI), decreased gastric mobility resulting in severe constipation, thrombophlebitis, and autonomic dysreflexia for lesions above T6[75, 89, 91]. No extraneous delivery complications are known to solely effect the SCI population, with the exception of the requirement to control severe hypertension for medically unmanaged autonomic dysreflexia[91].

It is important to note that women with clinically complete (according to criteria of the International Standards for Neurological Classification of SCI by the American Spinal Injury Association—ASIA) and anatomically complete injuries can sense cervical and vaginal stimulation, menstrual discomfort, and orgasm. These reported sensations implicate an alternate non-spinal pathway, the vagus nerve, as a potential source of input to the brain[92]. Nucleus tractus solitarius neurons, which receive direct vagal input[93, 94], have been shown to be responsive to stimulation of the cervix, vaginal canal, and uterus in animal models[95, 96]. However, while a bilateral vagotomy was shown to affect the responsiveness of these brainstem neurons, only a complete transection of the spinal cord eliminated responses in the nucleus tractus solitarius[96, 97]. Neurons in the nearby nucleus gracilis have also been shown in rats to respond to the stimulation of the clitoris and internal female reproductive organs, with responses varying throughout the estrus cycle[98-100], as well as in their subsequent target, neurons in and around the ventrobasal complex of the thalamus[101, 102].

Assessment of Sexual Function after SCI

Assessment of males with SCI. Currently, the most widely used method of assessing sexual function in human males is the International Index of Erectile Function (IIEF) [103-105]. The IIEF is a self-administered questionnaire consisting of 15 questions divided among five domains, including erectile dysfunction, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. The six questions concerning erectile function, for example, are assigned a score of 0 to 5, for a total of 30 possible points. Scores 25 to 30 indicate no dysfunction, 19 to 24 indicate mild dysfunction, 13 to 18 mild to moderate dysfunction, 7 to 12 indicate moderate dysfunction, and 0 to 6 indicate severe dysfunction. Important to studying a SCI population, the IIEF examines the ability to achieve versus maintain erection as two distinct entities[103]. However, the IIEF lacks consideration of psychogenic versus reflexive erections, as well as anejaculatory orgasm in SCI men. In 2009, the Autonomic Standards Committee validated the IIEF as an appropriate tool for assessing sexual function after SCI in clinical trials and recommended considerations to include when using the IIEF in this population[106]. Many of the questions do however rely on the subject having a partner at the time when the survey is given, which often may not be the case given the generally young age of the SCI male population. Another assessment developed for use in several drug studies is the Erection Hardness Grading Scale, which is a descriptive measure on a four-point scale[105].

Currently, no questionnaire exists solely to measure ejaculatory ability in SCI men. Clinically, however, our knowledge of reflex circuitries allows researchers and physicians to determine intact sexual pathways and predict ejaculatory ability[106]. The bulbocavernosus reflex can be visualized by stimulation of the glans penis and recording responses of the BS muscle or anal sphincter via EMG[107]. The bulbocavernosus reflex has been shown to indicate an intact S2-S4 spinal cord[108]. The hip flexor response is a pathogenic reflex initiated by stimulating the sole of the foot leading to flexion of the hip that is typically seen in SCI individuals. This reflex, if present, suggests an uncompromised spinal cord immediately superior to S2-S4. The presence of both the bulbocavernosus reflex and hip flexor response indicates the individual is likely capable of ejaculation with assistance of PVS[109]. These spinal reflex assessments are therefore useful clinically as indicators of sexual prognosis and as a tool to aid in the selection of future treatment plans (e.g., PVS or EEJ).

The International Spinal Cord Injury Male Sexual Function Basic Data Set was created in response to the need for easily comparable international sexual function data pertaining specifically to SCI men. This data set is collected by the clinician and assesses interest in discussing sexual issues, sexual problems unrelated and related to SCI, psychogenic erection, reflex erection, ejaculation, and orgasmic function[21]. The data set includes a section regarding the use of medications for sexual function. It is necessary to know if survey questions are being answered with drug or device usage to enhance sexual function as this can influence interpretation of the degree of dysfunction.

Assessment of females with SCI. The two most common tools used to assess female sexual function are the Female Sexual Function Index (FSFI) and the Sexual Function Questionnaire (SFQ) [104]. The FSFI consists of six domains including desire, arousal lubrication, orgasm, satisfaction, and pain. Nineteen questions are posed with scores ranging from 0 or 1 through 5, with the lowest score possible being 2 and the highest being 36[110]. Though the FSFI's use in SCI clinical trials has been limited, the Autonomic Standards Committee has endorsed its use for sexual function assessment in SCI women[106, 111-115]. The SFQ consists of eight factors including desire, lubrication, cognitive desire, sensational desire, orgasm, pain, enjoyment, and partner ratings among 28 questions[116]. This questionnaire may be beneficial in assessing SCI women as it considers both reflexive (sensation) and psychogenic (cognitive) arousal; however, the SFQ's use has also been limited in SCI[117].

Another method for sexual function assessment approved by the Autonomic Standards Committee for SCI women is the use of vaginal photoplethysmography to measure vaginal vasocongestion[106]. This physiological measurement is taken by using an intravaginal probe emitting infrared light that reflects off the vaginal wall and is captured via a phototransistor. The direct current received is believed to report total vaginal blood volume, where the alternating current signal reports vaginal pulse amplitude[118]. Current belief is that vaginal pulse amplitude is the preferred measurement for assessing vaginal responses to arousing stimuli[106]. Sipski and colleagues have utilized vaginal pulse amplitude to study sexual function in SCI women, including determination of the effects on vaginal vasocongestion in drug

studies, on lesion level or completeness, and for multiple stimulation types[74, 76, 86, 87, 119, 120].

The International Spinal Cord Injury Female Sexual and Reproductive Function Basic Data Set is similar to the Data Set for men discussed previously. This data set addresses the individual's interest in discussing sexual issues, sexual problems not related to SCI, sexual dysfunction related to SCI, psychogenic genital arousal, reflexive genital arousal, orgasmic function, and menstruation[21].

Assessment of animal models of SCI. Several methods have been used to assess sexual function in animal models of SCI. *Ex copula* reflexes to test sexual function in SCI male rats can be elicited by stimulation of the urethra, penile sheath retraction, or electrical stimulation of the dorsal nerve of the penis (DNP). The response to such stimulation includes penile engorgement, dorsiflexion, and glans cupping, with rhythmic contractions of the BS and IC muscles, with a much shorter latency than seen in intact animals[121, 122]. In female SCI rats, this reflex can be generated by stimulation of the urethra, causing pudendal efferent and cavernosus nerve bursts and rhythmic vaginal contractions[121]. Pressure recordings from the corpus spongiosum during reflexes or mating paradigms in awake male SCI rats can be determined by use of telemetric pressure devices, giving a qualitative and quantitative assessment of penile erection[123]. Finally, in anesthetized animals, EMG of the perineal muscles, typically the BS, can be utilized to determine physiological responses in sexual reflexes[121]. A recent study that utilized BS EMG in a chronic contusion model determined the D3 dopamine receptor agonist 7-hydroxy-2-(di-N-propylamino)tetralin-facilitated ejaculatory reflexes[124].

Intervention for males with SCI

Current therapies for treating sexual dysfunction in SCI men include pharmacotherapy, physical aids, and surgical interventions.

Pharmacotherapy. Pharmacotherapy interventions to assist erectile ability include intracorporeal (IC) therapy and phosphodiesterase type 5 inhibitor (PDE5I) oral medications. IC therapy consists of an intracavernosal injection of a cocktail including papaverine, phentolamine, and alprostadil[125]. However, IC therapy is expensive and can cause bleeding and scarring, and its use as a treatment option has waned with the introduction of PDE5Is[4]. PDE5I medications that have been assessed for use in SCI men include sildenafil (Viagra®), vardenafil (Levitra®), and tadalafil (Cialis®). In a clinical trial examining these three PDE5Is in SCI subjects, rigid erection sufficient for penetration was reached in 85 % of sildenafil users, 74 % of vardenafil users, and 72 % of tadalafil users with all three medications achieving mean persistent erection times of approximately 30 min. Individuals with upper motor neuron lesions responded best, while individuals with lower motor neuron lesions were poor responders. All three medications saw a significant increase in erectile function and intercourse and overall satisfaction when assessed with the IIEF-15, although only sildenafil significantly increased orgasmic function and ejaculation[126]. Alternative studies using the IIEF15 have also shown significant improvement in ejaculation with both tadalafil and vardenafil[127, 128]. Additionally, tadalafil and sildenafil have been examined for long-term use and dose effectiveness in SCI males[129-131]. Recently, 4-aminopyridnine, or fampridine, an oral medication used in SCI individuals for spasticity control, was examined for its

effect on sexual function. SCI men reported significant increases in two domains of the IIEF: erectile and orgasmic function, an effect secondary to the functionality of the medication[112].

Physical aids. Physical aids currently utilized by the SCI population for sexual dysfunction include vacuum tumescence devices (VTDs) for erectile dysfunction and PVS/EEJ for an ejaculation. Previous studies of VTD use in SCI men reported increases in sexual satisfaction, with one study reporting 60 % of participants and 42 % of their partners seeing improvements[132, 133]. However, in a 2008 study where SCI men used VTD for 1 month followed by using sildenafil for 1 month, no men were satisfied with the VTD[134].

For an ejaculation after SCI, PVS is a first-line therapeutic approach performed by using a medical vibrator to activate the DNP which signals to the ejaculatory center activating the ejaculatory reflex[70]. The vibrator is placed either on the dorsal or ventral side of the glans penis, with the most effective amplitude and frequency being 2.5 mm and 100 Hz [135]. Studies indicate that 86 % of SCI men with lesions above T10 achieve ejaculation; however, with lower injuries approximately only 20% succeed, as it is necessary for the sympathetic ejaculatory pathway to be intact [136]. Non-responders to a single vibratory device may respond to stimulation of both the dorsal and ventral glans penis, prompting the invention of the Viberect-X3 (Reflexonic,Frederick, MD, USA) that uses a single device to stimulate both the dorsum and frenulum of the penis[137, 138]. A recent study using this device in ejaculatory SCI men with injury levels above T10 reported a 77% success rate[138]. If PVS is not a viable option, EEJ is the suggested next treatment

option. EEJ is performed by rectally inserting a probe that uses electrical current to stimulate the smooth muscles of the seminal vesicles and prostate, which induces seminal emission. Since EEJ does not utilize the ejaculatory reflex, this method of sperm retrieval is appropriate for all injury levels[135]. EEJ has a high success rate, with the largest EEJ study to date reporting 91.9 % of SCI individuals achieving ejaculation[136].

Surgical intervention. Prosthetic penile implants to assist with erectile function can be inflatable, semirigid, flexible, or semiflexible. These devices have moderate satisfaction rates, with one study reporting 41 % of implanted SCI men seeing sexual function improvements[139]. A more recent study reported that 83.7 % of SCI participants with a penile implant could participate in sexual intercourse[140]. However, with the rise of less invasive alternative therapies and a high risk of complications, the use of these devices has slowed[141].

Surgical sperm retrieval is the remaining viable option if PVS and EEJ have failed. In this procedure, sperm is retrieved from the testis, vas deferens, or the epididymis by aspiration, biopsy, or open surgery. In vitro fertilization is almost always necessary with this method, as minimal amounts of spermatozoa with low motility are retrieved. Raviv et al. recently reported successful sperm retrieval using testicular sperm extraction in 89.6 % of 106 attempts, with 32 pregnancies of which 20 ended in live birth[142].

A new procedure called TOMAX has been described as restoring tactile and erogenous sensation of the glans penis in SCI men with lesions below L2, as well as

men with spina bifida. The TOMAX procedure uses microneurography to join the divided DNP to the sensory ilioinguinal nerve, which serves as a bypass to restore penile sensation. This procedure can be done either unilaterally or bilaterally; however, as the dorsal penile nerve is partially responsible for reflexive erections and ejaculation, bilateral TOMAX may jeopardize these reactions. In unilateral procedure, SCI and spina bifida men retained erectile and ejaculatory ability, with five subjects reporting reflexive erection, where previously only psychogenic erection had been present. A total of 80 % of individuals reported unilateral glans penis sensation, which originally reported as groin sensation but matured to real penile sensation in 33 %. Individuals also reported significant increases in penile rigidity and satisfaction during masturbation, along with a greater ease of maintaining an erection and reaching orgasm[143].

Neuromodulation by sacral nerve stimulation (SNS) has seen a success in SCI individuals in regards to bladder and bowel function[144]. In addition to these results, further benefits in sexual function have been observed[145]. SNS involves the implantation of a neurostimulator device via the S3 foramen, which sends mild electrical impulses to the sacral plexus and affects the innervated pelvic viscera. Early studies of SCI individuals with complete lesions examining the efficacy of SNS on bladder control also found significant results in erectile function, with 29 of 33 men involved achieving a full erection with stimulator use[146]. Another study examining effects of SNS on concomitant pelvic dysfunction in incomplete SCI men reported median IIEF-5 (questions pertaining to erection) scores increasing from

15.6 preimplantation to 22 post-implantation. While two individuals required re-implantation, their IIEF-5 scores returned to 22 at the 40-month follow-up[147].

Intervention for females with SCI

Current research in treatment of sexual dysfunction after SCI in women is sparse and has had limited success. One study examining SNS in 17 women with sexual dysfunction and neurologiclower urinary tract symptoms included five women with incomplete SCI. Of all 17 women, 36.3 % showed improvements of sexual function on both the FSFI and the Female Sexual Distress Scale after implantation. FSFI median scores increased from 22.7 pre-implantation to 26.02 post-implantation[111]. Another study examining the effects of sildenafil on sexual function in SCI women found no significant differences between sildenafil and placebo groups when assessed with SFQ, the Sexual Quality of Life Questionnaire—Female, a Sexual Distress Question, or a Global Efficacy Question, though a prior laboratory study saw benefits from sildenafil use[117, 119]. Similarly, the spasticity control medication fampridine had no effect on sexual function in SCI females[112].

Conclusions

Sexual function is a high priority QoL issue among the SCI population. The current state of research regarding sexual dysfunction after SCI reveals the need for better assessment and outcome measures geared toward dysfunctions specific to SCI populations. Additionally, the development of better assessment tools in animal

research will allow more effective therapies to be developed. Finally, the lack of effective treatment options for the female SCI population should be addressed.

The penile dorsiflexion reflex

After SCI, loss of descending inhibition from supraspinal areas allows for reflexogenic erections in response to cutaneous stimulation of the genital region, if the injury level is above the sacral cord. A similar response can be elicited in male rats, called the dorsiflexion reflex, which is used as the standard for analysis of sexual function in a rat SCI model. The dorsiflexion reflex is initiated by pushing the prepuce covering of the glans penis down, exposing the glans. Shortly thereafter, the penis will become elongated and engorged, and a cluster of penile reflexes will occur. These clusters are characterized by erections, penile cups (penile tip flaring), and dorsal flexions of the penis. Each cluster may contain several cups and flexions of the penis. Dorsiflexions of the penis represent erections, and cupping represents ejaculation[148, 149]. During a 30-minute testing period, these clusters will occur at 1-3 minute intervals[150-152]. While this response may occur in intact rats, the latency to reflex is much shorter in SCI rats. A transection of the spinal cord allows for an increased latency to reach the dorsiflexion reflex, but does not affect the cluster intervals, therefore suggesting that while the initiation of this reflex is under supraspinal control, the rhythm of the pacing mechanism may reside at the level of the spinal cord[150-152].

Evidence exists that male sexual function requires a brainstem loop. Nuclei located in the medullary reticular formation (MRF) including the nucleus reticularis gigantocellularis have been shown to be involved in such a loop[26, 59, 122]. Using bilateral stimulation of the MRF with varying types of chronic and acute spinal cord lesions, Hubscher and Johnson found evidence of descending bilateral projections

between MRF and lower thoracic/lumbar male urogenital circuitry within the dorsolateral quadrant (DLQ) and the most dorsal aspect of the ventrolateral quadrant of the midthoracic spinal cord[122]. This pathway is likely in control of the coordination of perineal muscle contractions and disruption of this pathway may allow for the hypersensitivity of the dorsiflexion reflex seen after SCI. Such supraspinal circuitry is believed to originate in the nucleus paragigantocellularis[26, 59].. Furthermore, Hubscher and Johnson also located a bilateral ascending pathway from the male genitalia in the DLQ of the midthoracic spinal cord, further supporting the idea of a spino-bulbo-spinal circuitry[153]. Therefore, it is currently thought that damage to these descending and ascending pathways causes a hypersensitivity of the local circuitry in response to afferent DNP stimulation.

Telemetric monitoring of intracavernosal pressure in the mating rat

Monitoring pressure changes during erectile events has been challenging. Previous methods include in vivo assessment of intracavernosal pressure (ICP) in an anesthetized animal, in vitro tissue assessment of the corpus cavernosum, or pharmacologically induced erectile responses[154]. Such methods suffer drawbacks due to single time point analysis, the use of anesthesia, and a lack of quantitative measurement of penile responses. Additionally, there has previously not been any use of in copula mating behavior as a measure of sexual function in animal models of SCI. In intact animals, numbers of mounts, intromissions, and ejaculations, and latencies to reach these copulatory steps are often used as indicators of sexual function[149, 155]. Mounts, intromissions, and ejaculations can be visually observed by a trained observer due to stereotypical behaviors; however, due to the quick nature of rat copulation, observation alone may not be the most accurate report of erectile and ejaculatory function[123]. Additionally, non-contact erections are the current model for the psychogenic erection seen in the human male[156]; however, as the rodent non-contact erection is olfaction based, and the human psychogenic erection is visual and auditory based, the central pathways may differ between species[148]. In contrast, radiotelemetric monitoring of ICP or intraspongiosal pressure (ISP) of the penis allows ICP or ISP to be measured in an awake, behaving animal[156-158]. In a previous study, the use of the pressure transducer in an intact animal during copulatory behavior (both in copula mating behavior and non-contact erections) assisted in confirming the visual observations of erections, and therefore increased the accuracy and sensitivity of the behavioral tests[123]. Though such

devices allow for the collection of penile pressure changes in behaving animals, this method has seen minimal use in animal models of SCI[123, 157, 158].

Potential therapeutic target for recovery of sexual function after SCI

Activity-based locomotor training (LT) after SCI has shown a range of improvements of multiple systems in both humans and animal models[159-164]. Evidence suggests that LT allows for the optimization of the lumbosacral spinal cord by providing afferent sensory input to stimulate spinal networks in the absence of supraspinal control and thus induce plasticity with repetitive activation to improve function[165, 166]. Male clinical research participants undergoing LT after SCI have shown increases of urogenital function after ABT, including increases in sexual desire. (hubscher impact) Additionally, SCI research participants have anecdotally reported improved sexual function after LT, even years after injury. Though sexual dysfunction severely alters quality of life after SCI, such implications of sexual function improvements after LT have yet to be examined in an animal model of SCI[167].

Experimental Directions

This dissertation research seeks to further investigate the impact of SCI on male sexual function utilizing quantitative outcome measures in a pre-clinical contusion animal model. To that end, the effect of activity-based training on sexual function in a rat contusion model was first examined. E*x copula* sexual reflexes were tested utilizing the penile dorsiflexion reflex, as well as the bulbospongiosus EMG response to stimulation of the DNP. It was first hypothesized that activity-based training, specifically quadrupedal locomotor training, would improve sexual function as measured through *ex copula* sexual reflexes, with increased PDFR onset latency and decreased burst duration and time to BSM response to DNP stimulation. Secondly, kinematic analysis of the PDFR and telemetric monitoring of intracavernosal pressure during mating behavior testing was used to identify and characterize sexual function deficits relative to intact controls in groups of rats having different extents of T9 contusion injuries. It was hypothesized that latency of PDFR onset and ICP would vary in SCI rats from control, regardless of injury severity. Additionally, it was hypothesized that differences in sexual function would be measurable between SCI injury severity groups. These studies further establish innovative techniques for quantitatively assessing sexual function after SCI and provide a greater insights into the mechanisms of sexual dysfunction after SCI.

CHAPTER II

ACTIVITY-BASED TRAINING ALTERS PENILE REFLEX RESPONSE IN A RAT MODEL OF SPINAL CORD INJURY

Introduction

Spinal cord injury (SCI) causes severe neurological impairment with widespread motor, autonomic, and sensory deficits, along with multiple secondary health complications causing substantial impairment in quality of life. Deficits in sexual function are rated as a top priority quality of life issue within the SCI population[21]. Scores on sexual assessment scales such as the Sexual Interest and Satisfaction Scale or the Sexual Adjustment Scale have been shown to correlate with quality of life assessment scores[6, 8]. A very limited number of experimental studies have focused on sexual dysfunction post-SCI, despite its high priority among SCI individuals [167].

Approximately 12,500 new cases of SCI occur annually, with 80.7% of SCIs occurring in males. SCI males rate their overall sexual satisfaction lower than the female SCI population, with 46% and 69% of respective populations reporting sexual satisfaction[21]. Sexual function deficits among SCI males include erectile and ejaculatory dysfunction and decreased fertility[10]. Although easily initiated, erections post-SCI are often short-lived and lack the rigidity required for penetration[6, 8]. Ejaculation is also adversely affected, with 95% of males with

lesions aboveT10 reporting severe impairment[36-38]. Such impairments may include anejaculation, dribbling expulsion of seminal fluid due to dyssynergia of the bulbospongiosus (BSM), ischiocavernosus (ICM), and urethralis muscles, or retrograde ejaculation resulting from improper bladder neck closure[49, 50, 54]. Aside from anejaculation, fertility may be impaired due to hypogonadism, hypospermatogenesis, and necrospermia[39, 41, 42, 71]. The current study, using a pre-clinical animal model, focuses specifically on examining potential therapeutic benefits of activity-based training on erectile dysfunction, which is the foremost basis for reports of dissatisfaction with sexual life by SCI males[21].

Activity-based training (ABT), including weight-bearing stepping on a treadmill (locomotor training – LT), has shown a range of effects across multiple systems in both SCI humans and animal models of SCI[159-164]. It is currently postulated that LT allows for the optimization of the lumbosacral spinal cord by providing sensory input to stimulate spinal networks in the absence of supra-spinal control and thus induce plasticity with repetitive activation to improve function [164-166]. Typically, step, stance, or swim recovery seen post-training is specific to the trained task; however, previous studies have reported gains in urogenital function after LT post-SCI[168-171]. Additionally, SCI research participants have shown a significant increase in sexual desire post-LT as assessed by the International Index of Erectile Function (IIEF) [103]. Participants also reported increased rigidity and/or more persistent erections, though this type of information was not captured with IIEF questionnaires (quantitative pressure recordings were not performed). The authors suggest that limitations of the current clinical assessment measures may conceal

potential training effects[170]. (For additional discussion on current assessments for sexual function in the SCI population, see Steadman and Hubscher, 2016[167]).

Therefore, the current study examines the effect of daily 60-minute ABT on sexual dysfunction in a clinically-relevant rat SCI contusion model using two established conventional outcome measures – awake penile reflex assessments and BSM electromyography (EMG) responses to stimulation of the dorsal nerve of the penis (DNP) in a terminal urethane-anesthetized in vivo preparation. Specifically, in an awake SCI rat, the ex-copula penile reflex is easily initiated by retraction of the penile prepuce, without further phasic stimulation. Briefly, reflex clusters occur which are characterized by 1-5 or more erections, glans tip cupping, and dorsal flexions of the penis (or "flips") within 5-10 seconds, followed by a rest interval of 30 – 120 s[150-152, 172]. Erections are visualized by reddening of the penis, extension and distension of the glans, with glans tip cupping in intense erections, while "flips" are penile dorsiflexions which occur with or without extension of the glans [172]. As ex copula sexual reflexes can pertain to multiple aspects of sexual responses in the rat animal model, and these reflexes can be elicited through multiple different methodologies, we felt it best to use the term "penile dorsiflexion reflex" (PDFR) as it is all encompassing of this particular sexual reflex elicited in this particular method as a whole. Additionally, because testosterone levels are known to decrease after SCI[173, 174], levels were periodically examined to determine if there was an effect of ABT.

Methods

Animals

All animal procedures were performed to the National Institutes of Health guidelines and the protocols reviewed and approved by the Institutional Animal Use and Care Committee at the University of Louisville, School of Medicine (IACUC #14025). Adult male Wistar rats (~300 g) from several other studies (examining recovery of upper or lower urinary tract function and effects of ABT) were assessed for sexual function and pooled together for the current study, yielding an N of 98 in total (100 less two that that did not survive the spinal contusion). Note that all methods were the same across studies, except for the terminal day testing. All rats were housed individually on a standard 12-hour light/dark cycle. Functional and behavioral assessments (see below) were conducted at regular intervals throughout the study. The urinary tract outcome data will be published elsewhere.

Spinal Cord Injuries

The following procedures can be viewed in a recent video journal published by our lab[175]. Animals were first anesthetized using an intraperitoneal injection of ketamine (80 mg/kg, Ketoset®; Fort Dodge Laboratories, Fort Dodge, IA), and xylazine (10 mg/kg, AnaSed; Lloyd Laboratories, Shenandoah, IA). The surgical area was then shaved, and the eyes lubricated to prevent drying. The animals were placed in a prone position on a heating pad to maintain a body temperature of 36-37°C until the animal recovered from anesthesia. A rostral/caudal incision exposed the T7 to T9 vertebra, and the T8 lamina was removed to expose the T9 cord. Spinal clamps were secured to the T7-T9 spinal process to stabilize the spinal

column. The Infinite Horizons impactor device (Precision Systems and Instrumentation, LLC; Fairfax Station, VA) was then used [176] to generate a 215 kilodyne contusion injury. The musculature and subcutaneous tissue were closed using Ethicon 4-0 non-absorbable surgical suture, and the skin was then closed with Michel clips.

Post-surgical care

Immediately after closure, a topical antibiotic was placed on the incision site. Animals were then given a subcutaneous injection of saline solution (5 mL/100g) for rehydration and 0.1 mL of Penject® dual penicillin (The Butler Company, Columbus, OH) as a general prophylactic. Additionally, animals were given 0.3 mL of gentamicin (GentaFuse®; Butler Schein, Dublin, OH) for 5 days post-surgery to prevent bladder infection and 0.2 mL meloxicam (Eloxiject, Henry Schein, Melville, NY) for 3 days post-surgery for pain management. Animals' bladders were emptied manually using the crede procedure 3 times/day until the animals were efficiently voiding (reflexive given the extent of injury).

Group Randomization for ABT

Per our previous studies[168, 169, 175, 177], four groups of SCI rats were included consisting of a quadrupedal trained (QT) locomotor stepping weight support group, a forelimb-trained (FT) non-weight bearing exercise group, a non-trained (NT) but harnessed SCI control group, and a home-cage (HC) group that was only transported to the training room but received no further intervention. Pre-training randomization at two-weeks post-SCI (usually 12 rats are done at a time due to long duration of doing multiple daily one-hour training sessions) yielded the

following: SCI+HC (n=33), SCI+NT (n=20), SCI + FT (n=26), and SCI + QT (n=19). The uneven group numbers reflect the pooling from different studies, but all had the same procedures apart from the terminal experiment. Data from the two non-ABT control groups were combined (SCI+NT and SCI+HC = SCI) as no significant differences were found between these groups. For assessing the effectiveness of randomization, IH impactor force and displacement values were grouped post-hoc and compared. Furthermore, day four post-SCI urine residual volumes (an indicator of injury severity[178]) collected during manual crede were used along with the Basso-Beattie-Bresnahan open field locomotor test scores (BBB[179]) obtained on Day 14 post-SCI as additional indicators of pre-training randomization efficacy between SCI groups.

Activity-Based Training (ABT)

The following procedures can also be viewed in a recent video journal published by our lab [175]. Two weeks after SCI, animals began ABT using an Exer 3/6 treadmill (Columbus Instruments, Columbus, OH) modified with spring scales for body weight support. Animals were harnessed in a Lycra vest (Robomedica, Inc. Mission Veijo, CA) with spring clips attached at rostral and caudal ends. FT animals were harnessed in customized vests that allowed for the hindlimbs to be slightly and comfortably elevated as to not come in contact with the treadmill. QT and FT animals underwent 60 minutes of LT daily for 8 weeks. NT control animals were also harnessed but remained on a nearby platform (no ABT). Throughout the 60 min training period, animals' step at warm-up speeds that increase to an "adaptability speed". The animals then undergo a "retraining period" during which the previous

cycle is repeated. The animal spends a total of 30 minutes of the 60-minute training at the adaptability speed[169]. Throughout training, the trainer gives attention to paw placement and coordination, animal welfare, and provides body weight support to the QT animals. Animals were removed early from training if the animals did not adapt to the confinement of the harness and treadmill (rare occurrence).

Penile Dorsiflexion Reflex (PDFR)

The PDFR was performed once pre-injury (after prior handling and restraint exposure) and at two-weeks post-injury just prior to the start of the training period (designated as “Week 0” with respect to training time). To account for potential within-subject variability, two data points per rat were collected during training, twice at early training effect time-points (at weeks 2 and 4 – individual rat data averaged and designated as “Week 4”) and twice at later training time-points (at weeks 6 and 8 – individual rat data averaged and designated as “Week 8”). For PDFR testing, each awake animal was restrained in a soft cylinder cotton cloth having just one open end for exposure of the hindquarters and placed in a dorsal recumbency position. The prepuce was retracted to expose the penis. A timer was then set to record the latency to the onset of the initial PDFR. For penile movement to be considered PDFR, both penile engorgement and a dorsiflexion of the penis were required. Penile glans cupping also often accompanies a PDFR but is not considered a requirement for classification. Animals were timed for the first two PDFR events; if no events occurred within 20 minutes, the animal was considered negative for PDFR at that timepoint[180]. Throughout each PDFR event, the number of dorsiflexions (“flips”) and glans cupping events were recorded.

Bulbospongiosus (BSM) EMG

Terminal EMG recordings of BSM were performed in response to DNP stimulation. Under urethane anesthesia (1.2g/kg i.p.; cat. no. CAS 51-79-6; Acros Organics, ThermoFisher Scientific, Waltham, MA), a 27-gauge needle was used to implant an electrode wire (Cooner) in the BSM, one into the left and one onto the right BSM. Each wire was then passed into the muscle using a 23-gauge needle and secured with sutures. A dorsal exposure and incision through the gluteus superficialis and bicep femoris muscles was made to access the DNP on the left and right side. Each nerve was separated from the connective tissue and placed in custom-made bipolar electrode cuffs. Using two Grass Photoelectric Stimulus Isolation Units (Model PSIU6; one per nerve) and a Grass S88 stimulator (AstroMed Inc., West Warwick, RI), the stimulus intensity was initially set at 30 μA, 0.1 msec duration with trains of 14 pulses at 50 PPS, 100 msec train duration, 1 train/sec [45–49]. Threshold was determined by first response to bilateral DNP stimulation in the BSM. Threshold stimulus intensity was then doubled, and testing repeated. During bilateral DNP stimulation, the Cambridge Electronic Device (CED Micro31401; Cambridge, England) acquired and digitally filtered EMG analog signals. Data was then analyzed using Spike 2 software (CED v8; Cambridge, England).

Testosterone Levels - Urine Sampling and Enzyme-Linked Immunosorbent Assay

Measurement of testosterone was done using urine collected during 24-h metabolic cage (CLAMS system) behavioral testing (data to be published elsewhere) [168, 178]. Urine samples were then centrifuged for 10 min at 4472 x g to remove any particulate matter (fur, dander, food) and aliquoted. These samples

were used to determine urine testosterone levels via Testosterone Enzyme-Linked Immunosorbent Assay (ELISA) kit (cat. no. K032-H5, Arbor Assays, Ann Arbor, MI) at several timepoints: pre-injury, pretraining (week 0), midpoint (week 5), and end of training/prior to terminal EMG (week 9) for 78 of the 98 total animals, representing all groups. The testosterone detection range for the kit was 10,000-40.96 pg/mL. Samples were diluted 1:40 per manufacturer and then tested in triplicate in a 96-well plate. Provided samples, non-specific binding wells, and zero standard wells were loaded in duplicate. Samples were incubated with the provided testosterone antibody for 2 hours at room temperature with gentle agitation, then aspirated and washed 4 times. 3,3'5,5'-Tetramethylbenzidine (TMB) substrate was then incubated for 30 min at room temperature. Stop solution was added to the plate to halt the TMB reaction, and the plate was immediately read at 450 nm.

Histology of Lesion Epicenter

After EMG terminal studies, animals were administered a lethal dose of anesthesia and then perfused with saline exsanguination fluid followed immediately by a paraformaldehyde/heparin solution and the tissue processed as previously described[181]. The T6 to T13 spinal cord was removed and placed in 4% paraformaldehyde for 2-4 days at 4^{O}C. The cord was then placed in a 30% sucrose/phosphate buffer solution for at least 24 hours prior to being transversely sectioned at 20μm thickness and mounted onto slides. Tissue was then stained using the Kluver-Barrera method to visualize white and gray matter.

Spot Advanced software (Diagnostic Instruments, Sterling Heights, MI) and the Nikon E400 microscope were used to obtain measurements to quantify the

lesion epicenter[178, 182]. Per previous protocols, white matter was divided into dorsal columns, dorsolateral funiculus, ventrolateral funiculus, and the ventromedial funiculus, as well as being divided into left and right sides. Landmarks for division include the central canal, the medial edges of the dorsal horn, and the ventral horn tips. Total areas of gray and white matter at the epicenter were divided by the areas of normal gray and white matter at 1 mm rostral to the epicenter to calculate the total percent sparing.

Statistics

Analysis was performed using SigmaStat (Systat Software, INC, San Jose, CA) and SPSS Statistics (IBM, Armonk, NY). The Levene test for inequality was used to determine equality of variance. Repeated behavior tests, including PDFR, time between PDFR events, numbers of penile dorsiflexion and glans cupping events, and urine testosterone levels used two-way repeated measures (ANOVA) (fixed effects) to compare between treatment groups within subjects and between subjects. The Holm-Sidak method post hoc test was used for penile dorsiflexion reflex latency pre-injury versus post-injury, numbers of penile dorsiflexions (SCI n=41, SCI + FT n= 19, SCI + QT n=19), and urine testosterone (SCI n=10, SCI + FT n=6, SCI + QT n=6). Due to the nature of the data, animals who were negative for PDFR at any timepoint were removed from the data set for time between PDFR events (SCI n=50, SCI + FT n= 20, SCI + QT n=16). Bonferroni post hoc t test was used for penile dorsiflexion reflex normalized to SCI control baseline (SCI + FT n=26, SCI + QT n=19). No post hoc tests were necessary for numbers of glans cupping events (SCI n=41, SCI + FT n= 19, SCI + QT n=19) or time between PDFR

events (SCI n= 50, SCI + FT n= 20, SCI + QT n=16). Terminal electromyography tests such as BSM response latency to stimulation of the DNP, average amplitude, and maximum amplitude used the Kruskal-Wallis One-way ANOVA on Ranks and burst duration and terminal weight analysis used One-way ANOVA with the Holm-Sidak method post hoc test. Pearson Correlations were used for penile dorsiflexions and glans cupping events versus bilateral percent dorsolateral white matter sparing. All values reported are mean ± standard deviation unless otherwise noted.

Results

In this study, measures of sexual function were examined after SCI and 8 weeks of ABT utilizing PDFR, BSM EMG to stimulation of the DNP, and urinary testosterone levels. A total of 98 male Wistar rats were tested for PDFR prior to receiving a moderate-tosevere SCI. Impactor measurements detected and recorded within the Infinite Horizon program at the time of contusion injury are presented in Table 1. Animals were given a two-week recovery period prior to onset of ABT. Collection of Day 4 post-SCI urine residual volumes and Day 14 over ground BBB locomotor scoring were conducted prior to starting of the eight-week one-hour daily training period. Post-hoc analysis testing for efficacy of group assignment randomization reveals no significant differences between groups at the onset of training ("Week 0") - for impactor parameters (force and displacement), residual urine volumes and BBB (Table 1).

LT alters penile dorsiflexion reflex after SCI

For e*x copula* reflex testing prior to SCI, the latency to onset of PDFR exceeded the 20 min testing period in 97 of 98 rats (one rat had a 12.57 min reflex latency). At two-weeks post-SCI prior to the beginning of ABT, the time to initiate PDFR was significantly reduced relative to preinjury baseline latencies ($F=626.9$, $df=1,8262.5$, $p< .001$). Additional post-hoc analysis testing for efficacy of group assignment randomization using the Holm-Sidak multiple comparison method revealed no significant differences between groups at the start of training. All three groups, however, had significantly decreased onsets of PDFR at baseline Week 0

(SCI p<.001; SCI + FT p<.001; SCI + QT p<.001) relative to pre-injury PDFR onset latencies (Figure 1A).

To examine the effect of ABT on the latency to onset of PDFR, animals were normalized to SCI controls at baseline (Week 0) as percentage of baseline control (latency of individual/pretraining SCI control latency) *100). The main effect of training group was significant such that SCI + QT animals had increased latency to onset of PDFR (F=24.5, df=1,122.8, p<.001).

Although there was no significant change in latency to onset of PDFR between groups at Week 0, SCI + QT animals had significantly increased PDFR onset latencies compared to SCI + FT animals at both Week 4 and 8. These data show a modest, but significant, trend towards PDFR normalization in the group subjected to hindlimb stepping with weight support (Figure 1B).

SCI but not ABT impacts the number of penile dorsiflexion events

During the *ex-copula* reflex testing, the onset of the first PDFR event and the onset of the second PDFR event were recorded. The time between PDFR events was examined to determine role of ABT on the refractory period between PDFR events. Animals who tested negative at any time-point of the bi-weekly testing (i.e. PDFR latency of 20 min) were excluded from this analysis, as they did not have a refractory period to be examined. There were no significant changes in the time between PDFR events between weeks or groups (SCI n=50, SCI + FT n=20, SCI + QT n=16) (Figure 2A).

The number of penile dorsiflexion (“flips”) and glans cupping events were recorded during the PDFR *ex copula* test in a subset of the animals (SCI n= 41; SCI

+ FT n= 19; SCI + QT n= 19). There were no significant differences in penile flip events between groups; however, the number of penile flip events was significantly elevated at Week 4 (p= .006) and Week 8 (p= .023). (Figure 2B). No significant differences were found in number of glans cupping events between groups, between weeks, or between groups within weeks (Figure 2C).

Activity-based Training alters Bulbospongiosus Muscle EMG

After 8 weeks of ABT, EMG of the BSM to stimulation of the DNP was recorded in a subset of animals from this study (SCI n=24; SCI + FT n=12; SCI + QT n=12; see example in Figure 3A). Latency to onset of BSM response for the SCI + QT animals was significantly decreased compared to SCI controls (Figure 3B). Burst duration, measured per animal by averaging 10 successive EMG responses, was significantly shorter for ABT groups (both SCI + FT and SCI + QT, see Figure 3C). There was no significant difference between ABT groups and controls with respect to the average amplitude of the BSM response and the maximum amplitude (data not shown).

SCI but not ABT alters urine testosterone levels

Urine was collected via 24-h metabolic cage behavioral testing pre-injury, and post-injury at pretraining (Week 0), at Week 5 of ABT, and prior to terminal (Week 9). A subset of collected urine was used to run a testosterone ELISA. Urine testosterone level was shown to be significantly higher pre-injury ($F=12.109$, $df=3,76852121$, $p<.001$) compared to post injury. Urine testosterone was significantly lower relative to pre-injury at Week 0, Week 5, and Week 9. There were

no other significant changes in urine testosterone between post-injury weeks, between groups, or between weeks within groups (Figure 4).

Animal Terminal Weights and Lesion Histology

Rats receiving 8 weeks of one-hour daily ABT weighed significantly less than their SCI control counterparts at the time of the terminal EMG experiment (Table 2). Histological examination of the lesion site revealed a complete loss of grey matter at the lesion epicenter with a rim of spared white matter (WMS). There were no significant differences in overall bilateral WMS between groups. There were also no significant differences between bilateral white matter sparing (WMS) of either the dorsolateral quadrant (DLQ - where descending inputs mediating sexual function are located) or the ventrolateral funiculus (VLF - Figure 6).

Discussion

The PDFR occurs more frequently in spinally lesioned rats and when it occurs in spinally intact rats, there are fewer dorsiflexions of the penis and less intense cupping of the glans[151]. In the current study, prior to injury only 2% of the animals had a PDFR onset latency less than the 20 minutes allotted to induce the reflex. The basis for this lack of occurrence stems from existing bilateral descending inhibitory projections from the gigantocellularis and lateral paragigantocellularis nuclei in the medulla to the lumbosacral circuitry responsible for control and coordination of the perineal musculature[23, 26, 59, 122, 183-185]. Specifically, activation of the BSM evokes glans engorgement and cupping (erection) and activation of the ICM evokes dorsiflexion of the penile body[25, 186, 187]. Pudendal motoneurons innervating the BSM and ICM originate at the L6-S1 dorsomedial nucleus and the L5-L6 dorsolateral nucleus in the ventral horn, respectively[188, 189]. Therefore, at two weeks post-SCI, in the absence of descending reticulospinal projections (previously shown to reside in the dorsolateral quadrant of the T8 spinal cord[122]) the PDFR was easily induced in *all* rats, with a relatively short latency to onset.

After undergoing eight weeks of ABT, only the SCI + QT group of animals showed a significant increase in the latency to onset of PDFR, as early as 2-4 weeks post-training. Benefits from weight-bearing/limb placement/sensory input to the lumbosacral cord below lesion-level have been previously demonstrated in both animal and human studies showing autonomic (non-locomotor) improvements after training, including bladder function[168-171, 190], cardiovascular function[162, 191],

and kidney function[168]. Additionally, human clinical research participants undergoing weight-supported stepping on a treadmill after SCI have shown improvements in sexual function where there was a significant increase in sexual desire (anecdotal reports of improved erectile function were not detectable in questionnaire scoring using the International Index of Erectile Function) [170].

The mechanism underlying this small but significant change in latency is not clear but includes the possible strengthening of small residual descending projections traversing the contusion injury within the rim of spared white matter present at the lesion epicenter. Current ongoing studies include experiments on SCI animals with different lesion extents. Note that this latency change from post-SCI baseline was not found for the SCI + FT trained group of animals, suggesting the effect was not exercise-mediated or metabolically induced. This finding is different from our results of ABT effects on polyuria, which showed significant improvements in both SCI + QT and SCI + FT groups, likely reflecting changes in circulating hormones involved in fluid regulation that are known to be affected by exercise[168].

For rat copulation, loss of modulatory control of the ICM impacts intromission during mating and penile dorsiflexions during PDFR[186, 187]. Likewise, loss of modulatory control of the BSM leads to less intense erections (similar to what is found in humans), no glans cupping, and decreased fertility[187, 192]. Glans cupping is necessary for mating and fertility in that it is critical for appropriate ejaculation and placement of the seminal plug. As stated above, penile dorsiflexion and glans cupping during the ex-copula penile dorsiflexion reflex are under descending brain stem control. Indeed, lesion of the nucleus paragigantocellularis

(nPGI) results in increases in number of penile dorsiflexions, though there are no changes in glans cupping[26], findings consistent with the SCI effects in the current study.

After the initial PDFR event, subsequent PDFR events followed in one to three-minute intervals (see Figure 2) with continued prepuce retraction[151]. Previous studies have shown similar interval clusters (time between PDFR events) in mid-thoracic spinally transected rats, supportive of a spinal pacemaker in the cord below the level of lesion[150, 152] and consistent with findings that lesions of the nPGI do not alter the time between PDFR events[26]. A spinal ejaculation generator (see Allard et al 2005 for review) [193] at L3-4 contributes to the integration and coordination of the sympathetic, parasympathetic, sensory inputs, and motor outputs of the sexual reflex[36, 124, 193-196]. In this study, PDFR intervals were unchanged by ABT, which could reflect a persistent timing mechanism not impacted by below-level activity-based plasticity.

In the current study, penile “flips” changed over the course of the eight weeks post-injury; however, there was no training effect and no correlations with DLQ WMS. Considering the slight recovery of the PDFR onset latency in the SCI + QT group, a decrease in number of penile “flips” would be expected but may not have been detected due to the lack of sensitivity of the measure to discern changes in strengthened residual connections or increases below-lesion synaptic plasticity. Alternatively, a contusion injury may leave enough residual bilateral connections spared that the number of penile “flips” is already a “recovered” amount as compared to the numbers that would be seen after a complete disruption in the

descending inhibition through either lesion of the nPGI or complete transection of the spinal cord[26, 122]. Glans cupping events were not changed throughout weeks or within groups, with no correlation with DLQ WMS. Again, this aspect of the PDFR may not be appropriate for discerning subtle circuitry changes. However, as glans cupping is not altered by nPGI lesion, it has been postulated that descending inhibition of penile erection and glans cupping are dependent on local circuitries[26].

Activation of the DNP, sensory afferents necessary for erectile and ejaculatory reflexes [23, 167, 197, 198], causes rhythmic bursting of the BSM as seen during ejaculation in both human and rat[199-201]. BSM response to stimulation of the DNP is difficult to induce in a spinally intact rat, though as with PDFR it is easily elicited in a transected animal[199, 202]. Tonic descending inhibition from medullary brainstem centers is believed to be responsible for inhibition of this reflexic BSM response in the intact rat[26, 122]. In this study, we examined the BSM response to DNP stimulation in the urethane-anesthetized male rat after undergoing 8 weeks of ABT. SCI + QT animals exhibited shorter latencies to onset of BSM activity, suggesting weight-bearing stepping may strengthen the sexual reflex circuitry between the DNP and lumbosacral spinal cord. BSM bursts of SCI + QT and SCI + FT animals had a significantly shorter duration, which may be an effect of exercise in general. The presence of some spared long ascending and descending propriospinal pathways responsible for interlimb coordination can alter neural circuitry at the lumbosacral level, providing a potential mechanism through which forelimb exercise may affect BSM burst duration. In relation to the PDFR, decreases in latency and burst duration suggest increased local reflex

synaptic efficacy, where the longer onset of PDFR suggests increased descending tonic inhibition.

Serum testosterone is known to decline after SCI and may play an additional role in sexual dysfunction apart from neurological deficits[173, 174, 203-205]. After SCI in the current study, rats had a significant decrease in urine testosterone levels that remained depressed for the duration of the study. Thus, testosterone levels are not likely a contributing factor for the altered sexual reflex functions occurring with ABT. In contrast, male SCI research participants undergoing activity-based training (arm-crank exercise) have a significant increase in serum testosterone after 12 weeks of training, and there is a significant inverse correlation with waist circumference and testosterone levels[206]. Such changes in testosterone levels post-training were not found in the current study even though trained animals weighed significantly less than their SCI counterparts at the time of terminal experiments (no significant correlation between weight and testosterone). These differences may be due to differences in methods and time of collection.

In summary, sexual dysfunction after SCI remains a top priority to SCI individuals, while remaining one of the least studied areas of post-SCI animal research. The current state of the field is partially due to a lack of outcome measures and assessment tools geared towards sexual dysfunction after SCI[167]. In human SCI studies where participants underwent ABT, participants reported an increase in sexual desire, but there were no significant changes in erectile rigidity per the International Index of Erectile Function. However, multiple participants reported longer lasting erections and/or increased penile rigidity after undergoing

ABT, and this may be a limitation of available assessments for measuring changes in erectile function[170]. Animal models of sexual function after SCI are hindered by limited efficient mechanisms for assessing erectile function, with past studies utilizing *ex copula* reflexes (as seen in this study), *in vitro* tissue assessment, or pharmacologically induced erections, which all suffer from various drawbacks including anesthetic use, single time-point analysis, and subjective quantitative measurement of penile responses[154]. Such limitations in assessment tools may be overcome, for example, in both humans and animal models by use of pressure recordings [123, 157, 207]. While much quantitative data has been derived from previous studies of *ex copula* sexual reflexes [150-152, 186, 187, 192, 208, 209],advances in technology in telemetric pressure transducers allows for hyper-detailed quantitative measurements over chronic time-points. Animal models of sexual function have seen success with telemetric pressure transducers for examining penile pressures (both intracavernosal and intraspongiosal) during *ex copula* reflexes, as well as real-time mating behavior[123, 157, 207, 210]. However, mating behavior after chronic SCI has yet to be examined using telemetric pressure transducers and is the focus of our current ongoing study. Utilization of such methods in animals undergoing ABT after SCI may elucidate further training effects.

Results from this study also reveal potential interactions of neural circuitries that converge within the lumbosacral cord that may be used as an indirect conduit to affect each other and perhaps be used to impact sexual function after SCI. Tibial nerve stimulation has previously seen success in treatment of detrusor hyperreflexia after SCI [211]. Our results suggest LT may have a positive influence on sexual

function after SCI, providing a potential avenue to explore whereby the efficacy of current sexual dysfunction therapies may be enhanced.

Potential mechanisms whereby training impacts upon sexual reflexes after SCI may include propriospinal and other afferent input as well as local spinal circuities which are the main drivers of the autonomic and motor output of the spinal cord [170, 212, 213], potentially yielding plastic alterations within the lumbosacral cord. Additionally, modest recovery in these pudendal-pudendal reflexes may also result from the strengthening of spared descending pathways allowing for a return of some limited medullary descending inhibition, as intense LT has been shown to increase the connectivity of spared corticospinal pathways [214]. Other improvements in the SCI+FT exercise-only animals may be explained by strengthening of interlimb propriospinal circuitries from repetitive coordinated forelimb-hindlimb movement impacting functional reorganization within the lumbosacral cord[215-218].

Conclusions

ABT provides sensory input via task-specific stepping and/or hindlimb loading to the spinal cord which thereby positively impacts the sexual function circuitry in a rat SCI contusion model. Such potential effects of ABT warrant further investigation as a therapeutic target with which to improve sexual function in men with SCI.

Table 1

SCI IH Impactor Parameters and Pre-Trainng Assessments

Group	# Rats	Injury Force (kdyne)	Displacement (μm)	4 Day Urine (mL)	Pre-Training BBB
SCI	53	227.8 ± 24.7	1411.6 ± 238.9	2.54 ± 1.4	8.9 ±1.9
SCI + FT	26	229.4 ± 29.4	1399.9 ± 139.3	2.51 ± 1.6	9.1 ± 1.4
SCI + QT	19	230.4 ± 35.1	1447.7 ± 134.9	1.96 ± 1.1	9.1 ± 1.7

No significant differences; $p>.05$. Values are means ± standard deviation.

Table 2

Terminal weight

	Terminal Weight (g)
SCI (n=53)	483.6 ± 52.3
SCI + FT (n=26)	432.8 ± 50.7*
SCI + QT (n=19)	419.9 ± 42.5*

*p<.001. Values are means ± standard deviation.

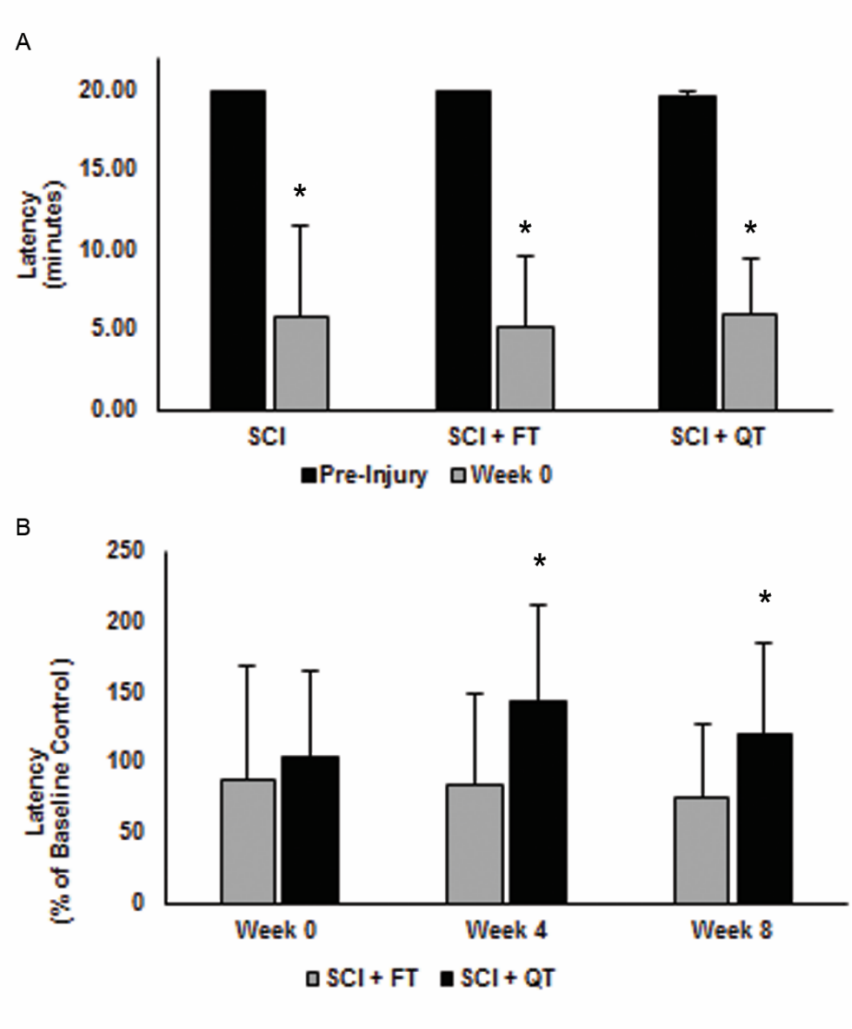

Figure 1 Latency to onset of PDFR.

A) Latency to onset of PDFR exceeded the 20-minute testing period pre-injury. PDFR onset was significantly decreased in SCI control (n=53; ∗p<.001, SCI + FT (n=26; ∗p<.001), and SCI + QT (n=19; ∗p<.001) groups post-Injury prior to ABT (Week 0). B) Latency to onset of PDFR normalized to SCI control baseline showed a significant difference between SCI + FT (n=26) and SCI + QT (n=19) groups at Week 4 (∗p<.001) and Week 8 (∗p=.017). ABT – activity-based training; FT - forelimb trained; PDFR - penile dorsiflexion reflex; QT - quadrupedal trained; SCI - spinal cord injury. Error bars are mean ± standard deviation.

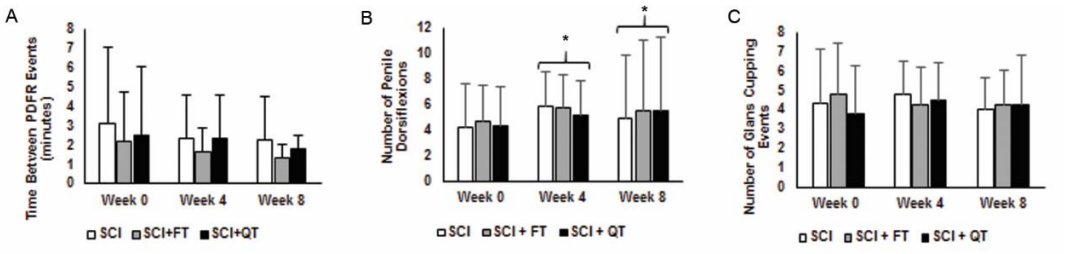

Figure 2 Time between PDFR events and penile dorsiflexion and glans cupping. Time-interval between the PDFR's (SCI control n=50; SCI + FT n=20; and SCI + QT n=16) and total penile dorsiflexion and glans cupping during two successive PDFR's (SCI control n=41; SCI + FT n=19; and SCI + QT n=19) was recorded in a subset of animals. A) The time between the first PDFR event and the second PDFR was not significantly different between groups or across weeks. B) The number of penile dorsiflexions were significantly greater in all groups compared to Week 0 at Week 4 ($p = .006$) and Week 8 ($p = .023$) C) The number of glans cupping events did not change over the 8-week testing period. There were no significant differences between training groups at Week 0, Week 4, or Week 8. Error bars are mean ± standard deviation.

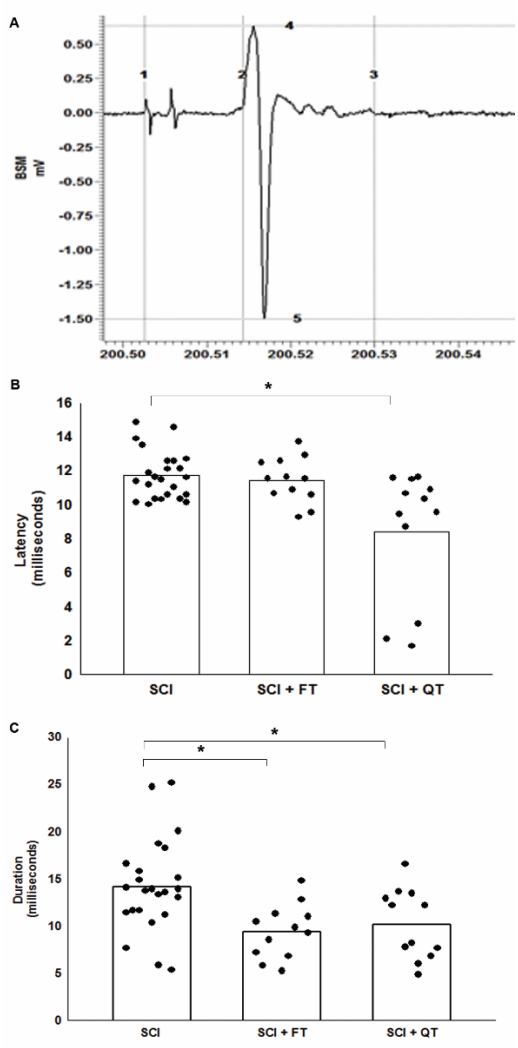

Figure 3 BSM EMG responses to stimulation of the DNP.

EMG response measurements (A) indicating response latencies (where 1 denotes stimulation artifact onset and 2 denotes onset of EMG response), EMG response duration (where 2 denotes onset of burst and 3 denotes end of burst), and response amplitude (between lines 4 and 5). EMG BSM response latency to stimulation of the DNP (B) was significantly shorter in SCI + QT (n=12; $*p<.05$) animals compared to SCI non-trained controls (n=24). The burst duration of both training groups (SCI+ FT, n=12 and SCI + QT, n=12) was significantly shorter compared to SCI non-trained controls (n=24; * $p<.01$).

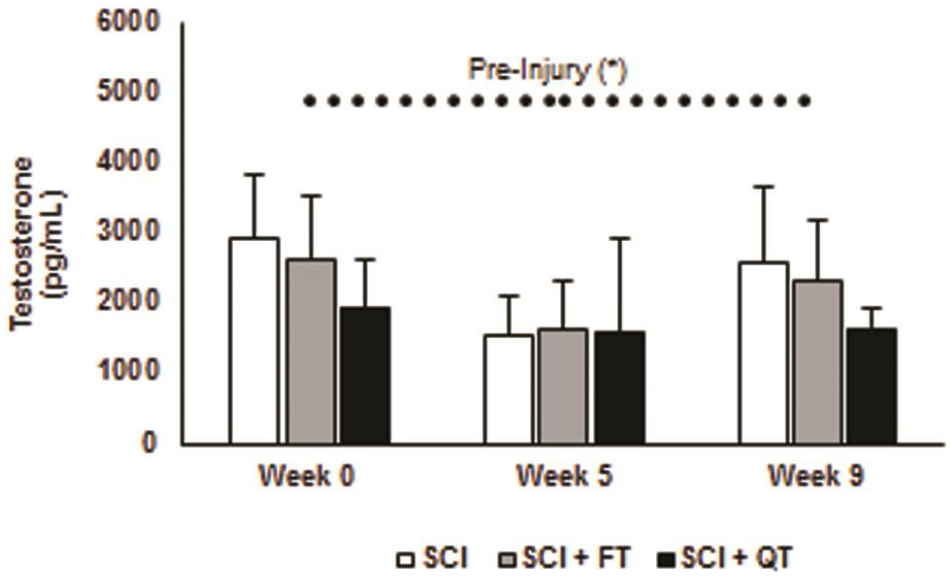

Figure 4 Urine testosterone levels.

Urine testosterone levels were determined using a subset of animals (n of 10 for SCI, n of 6 for SCI + FT, and n=6 for SCI + QT. Pre-Injury urine testosterone levels were significantly higher ($*p<.001$) than urine testosterone levels at Weeks 0, 5 and 9. There were no significant differences between post-injury time points or between training groups. Error bars are mean ± standard deviation.

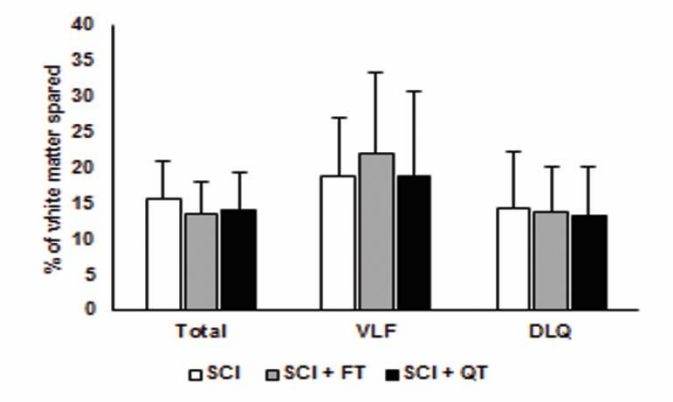

Figure 5 White matter sparing.

There were no significant differences of total white matter sparing, bilateral ventral lateral funiculus (VLF) white matter sparing, or bilateral dorsolateral quadrant (DLQ) white matter sparing between groups. Error bars are mean ± standard deviation.

CHAPTER III

KINEMATIC ANALYSIS OF PENILE REFLEXES IN A RAT MODEL OF SPINAL CORD INJUY

Introduction

Spinal cord injury (SCI) results in widespread multi-system neurological impairment that includes motor, autonomic and sensory deficits. One deficit, sexual dysfunction, is a high priority quality of life issue for SCI individuals. Scores from quality of life assessments directly correlate with scores from sexual assessment scales, including the Sexual Interest and Satisfaction Scale and the Sexual Adjustment Scale[6, 21]. Such deficits in sexual function in the male SCI population include erectile and ejaculatory dysfunction and impaired fertility[21]. Short-lived erections with insufficient rigidity occur[8, 36], as well as anejaculation or dribbling ejaculation due to dyssynergia of the bulbospongiosus (BSM), ischiocavernosus (ICM) and urethralis muscles and retrograde ejaculation due to improper closure of the bladder neck[40-42]. Despite the high priority level amongst SCI males, few experimental studies have focused on sexual dysfunction in a relevant pre-clinical animal model. Currently, a shortage of sensitive measures for human sexual function, as well as in pre-clinical animal models contributes to the gap in research[167].

One measure of sexual function in the animal model is the *ex-copula* penile dorsiflexion reflex (PDFR). In the rat, mechanical retraction of the prepuce may trigger the PDFR which consists of engorgement of the penile body, penile glans tip cupping, and dorsiflexion of the penile body[152]. Glans engorgement and cupping is due to activation of the BSM (whose motoneuron pool resides in the L6-S1 dorsomedial nucleus), while penile body dorsiflexion is due to activation of the ICM (whose motoneuron pool resides in the L5-L6 dorsolateral nucleus) [186, 187, 219]. The PDFR is difficult to elicit in spinally intact male rats[150] due to tonic descending inhibition from supra-spinal brainstem centers[26, 59, 122] but is easily evoked after spinal transection[122, 150, 186].

Disruption of bilateral descending reticulospinal projections reduces the synaptic efficacy of the dorsal nerve of the penis (DNP) onto motoneurons controlling the perineal musculature. This desensitized circuitry allows for a hyper-excitable state of reflex activity[26, 122]. After spinal cord lesion, the latency to onset of the PDFR is significantly reduced, with increases in numbers and intensity of penile dorsiflexion and glans cupping as compared to non-injured animals[150, 220]. Injury-induced alterations in sexual reflex circuit excitability allows for a quantifiable measure of sexual function; however, there is limited knowledge on the state of the circuitry where residual fibers remain traversing the injury (i.e. with a more clinically-relevant contusion injury rather than a complete spinal transection which rarely occurs clinically). Therefore, the purpose of this study is to assess if there is a relationship between the sexual reflex response and injury severity, using kinematic analysis of the PDFR to detect potentially subtle changes in the erectile response.

Methods

Animal Care

All procedures were carried out to the National Institutes of Health guidelines and protocols were reviewed and approved by the Institutional Animal Use and Care Committee at the University of Louisville, School of Medicine. Twelve adult male Wistar rats (~300g) were individually housed on a standard 12-hour light/dark cycle. To insure a range of incomplete injury extents, the animals were randomized to one of three T9 level contusion severity groups; a 150 kilodyne (n=4), 175 kilodyne (n=4), or 215 kilodyne (n=4) injury force, performed using an Infinite Horizons Impactor (Precision Systems and Instrumentation, LLC; Fairfax Station, VA) [176]. The Basso, Beattie, and Bresnahan (BBB) scale was used to assess over ground locomotion preoperatively, postoperatively, and prior to sacrifice[179]. PDFR kinematic testing was performed once prior to injury (after handling and habituation to restraint) and at weekly intervals beginning at week three post-injury.

Spinal Cord Injury

Surgery. The following procedures can be viewed in a recent video journal published by our lab[175]. Animals were anesthetized using an intraperitoneal injection of ketamine (80 mg/kg, Ketoset®; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (10 mg/kg, AnaSed; Lloyd Laboratories, Shenandoah, IA). Each animal was prepared for surgery, injected subcutaneously with 5 ml of sterile saline solution, and placed in a prone position on a heating pad to maintain a body temperature of 36-37^{O}C. The T7-T9 vertebra were exposed via a rostral/caudal

incision and the T8 lamina was removed to expose the T9 spinal cord. The spinal column was stabilized using spinal clamps attached to the T7 and T9 processes. Incomplete lesions were made using the Infinite Horizons impactor device followed by closure of the muscle and subcutaneous tissue using 4-0 Ethicon non-absorbable surgical suture and Michel clips for skin closure.

Post-surgical care. A topical antibiotic (bacitracin) was placed on the wound immediately after closure. Animals then received another 5 mL of subcutaneous saline solution for hydration, 0.1 mL of Penject® dual penicillin (The Butler Company, Columbus, OH) as a general prophylactic, and 0.3 mL of gentamicin (GentaFuse®; Butler Schein, Dublin, OH) to prevent bladder infection. The gentamicin regimen was continued for 5 days post-surgery, and 0.2 mL of meloxicam (Eloxiject, Henry Schein, Melville, NY) was also given twice a day for 3 days post-surgery for pain management. Per established protocols[168, 169, 175, 178], animals' bladders were emptied 3 times/day using the crede procedure until the animals were reflexively voiding without assistance. Animals had a two-week recovery period prior to any behavioral testing. At fourteen-day post-SCI, the BBB locomotor assessment was used as an early assessment of injury severity. PDFR testing was performed weekly for 6 weeks post-injury after completion of the two-week recovery.

Penile Dorsiflexion Reflex (PDFR)

Awake animals were placed in a soft cylinder cotton cloth with hindquarter exposure and placed in a dorsal recumbency on a platform. The prepuce was then

retracted to expose the penis. A set timer was used to record the latency to the onset of the initial PDFR and one subsequent PDFR. Penile dorsiflexion of the penis was required for penile movement to be considered a PDFR response. Glans cupping often accompanies penile dorsiflexion but is not required for PDFR classification. Animals were timed for two PDFR events; if no PDFR event occurred within 20 minutes, the animal was considered negative for the reflex at that time point[192]. Throughout both PDFR events, the number of dorsiflexions (penile “flips”) and glans cupping events were recorded.

PDFR Kinematic Recording and Analysis

Kinematic analysis was performed weekly after a two-week recovery period post-injury. Permanent markers were used to place point of reference dots on the platform for consistent measurements between animals. Prior to beginning the PDFR test, point of reference markings were placed on the skin superior to the prepuce and on the glans penis. PDFR testing was recorded from a sagittal viewpoint using highspeed video with a capture rate of 100 frames/second. Video analysis was performed using MaxTraq software (Innovision Systems, MI, USA). Animals were recorded for two PDFR events; if the animal was negative for a PDFR, no event was recorded.

Kinematic analysis of the PDFR using markings on the platform and animal examined three elements: maximum angle of penile dorsiflexion (MAPD), total penile event duration (TPED), and penile ascent speed (PAS). A schematic representation is presented in Figure 6. MAPD was measured using marks placed at the base of

the prepuce, tip of the glans penis, and permanent relation platform marks. Analysis began at the first penile movement from rest position. The MAPD was determined to be the maximum calculated angle created by the vectors of 1) the two permanent platform marks (points E and F; Figure 6) which spanned 5 cm in a straight line, and 2) the vector created by the marks placed at the glans tip and base of the penis (points D and A-C; Figure 6) during a penile dorsiflexion. TPED was calculated as the time of the full penile dorsiflexion event, from first movement from rest position, to return to rest. PAS was determined from the distance (cm) traveled from first movement from penile rest position until the maximum angle was reached and the time (sec) that it took for this to occur. It is common for the penis to remain at the maximum angle for several frames (i.e., several msec). During PDFR testing where more than one penile dorsiflexion occurred during the two recorded PDFR events, measures were calculated as an average of all individual MAPD, TPED, or PAS measures.

Histology of lesion epicenter

After final PDFR testing, animals were administered a lethal dose of anesthesia and perfused with saline exsanguination fluid immediately followed by a paraformaldehyde/heparin solution and the tissue processed as previously described[181]. Briefly, the T6 to T12 spinal cord was removed post-perfusion and placed in a 4% paraformaldehyde solution for 2-4 days at 4C. Twenty-four hours prior to sectioning, the tissue was moved to a 30% sucrose/phosphate buffer solution. The tissue was then transversely sectioned at 20um thickness on a cryostat

and mounted onto slides. The Kluver-Barrera method was then used to stain the tissue to visualize the white and gray matter.

A Nikon E400 microscope and Spot Advanced software (Diagnostic Instruments, Sterling Heights, MI) was used to capture tissue images for analysis and obtain measurements to quantify lesion extent. As previously described[178, 182], total white matter sparing was assessed based upon intact areas averaged from above and below the level of injury at the lesion epicenter. The left/right white matter sparing was further assessed by sub-regions in a section from the region having the largest lesion volume: dorsal columns, dorsolateral funiculus, ventrolateral funiculus, and ventromedial funiculus. The central canal, the medial edges of the dorsal horn, and the ventral horn tips were used as landmarks to guide cord divisions.

Statistical Analysis

Analysis was performed using Excel (Microsoft Office, Seattle, WA) and SPSS Statistics (IBM, Armonk, NY). The Levene test for inequality was used to determine equality of variance. PDFR testing was first examined on a weekly basis. Due to the nature of the PDFR testing, animals who were negative at any timepoint were not included for that specific timepoint (i.e. if animal was negative for PDFR at week 3, they would only have 2 points of data for the early timepoint). The Binomial Proportions two-tailed test and the Spearman Rank-Order correlation test were used for post-hoc analyses of the kinematic data.

Results

In this study, the PDFR of twelve animals was examined by kinematic analysis. Prior to SCI, all animals were tested and 100% exceeded the 20-minute PDFR testing period. Weekly PDFR kinematic analysis began during the third week post-injury. Post-injury analysis of Infinite Horizon generated data revealed actual forces and displacements consistent with two clusters of injury severities reflecting moderate and moderate-severe extents (characterization terminology labels used are based upon perceived level of functional deficits), except for one animal outlier per group. WMS and two-week BBB for those two animals met outlier test criteria, which was used as justification for group re-assignment. Note that post-hoc WMS was used to determine extent of injury (typical example provided in Figure 7) rather than IH impactor output force values at the time of injury.

The combined distribution of all individual animal kinematic data, including angle, event duration, and speed, is summarized in Figure 8. Based upon the median distribution of all data points (see horizontal line in each plot), MAPD, TPED, and PAS were allocated to high and low/no performer groups with respect to median split of the recorded measurements[221]. High performers are thus designated as large MAPD (greater than 71.06 degrees), long TPED (greater than 0.86 s), and high PAS speed (greater than 7.72 cm/s). In addition, there are observational trends in the data in early weeks (post-injury 3-5) relative to late weeks (post-injury 6-8), resulting in the separation of data based upon time after injury and thus subsequent analysis into 3-5- and 6-8-week time-points.

PDFR testing was analyzed at post-injury weeks 3-5 and post-injury weeks 6-8 time-points. At the early time points (post-injury weeks 3-5 combined), the number of moderate injury animals positive for a PDFR response was significantly fewer than the number of PDFR-positive moderate-severe injury animals ($p < .005$). Similarly, at the late time points (post-injury weeks 6-8 combined) the number of PDFR-positive moderate injury animals was significantly fewer than the number of moderate-severe injury animals presenting with a PDFR response ($p < .001$; Figure 9). There were no significant differences between time points in either injury severity group.

Injury severity affects MAPD, TPED, and PAS

Kinematic analysis of the PDFR allowed for the detection of the MAPD, TPED, and PAS. The MAPD was determined by the maximum angle reached by the angle formed between the vectors of the reference points and the base of the penis to the glans tip (see Figure 6). Median split was used to determine large (> 71.1°) versus small (≤ 71.1°) angles. The number of moderate injury animals with large angles was significantly fewer than the number of moderate-severe injury animals with large angles at the early time point ($p < .05$), as well as at the late time point ($p < .001$; Figure 10A). There were no differences within injury groups between time points.

TPED was determined by the duration of the penile event from first framed movement of rest to the point of return to rest. Median split was used to sort durations into long (> 0.86 s) and short (≤ 0.86 s) durations. At the early time point,

there were no significant differences between the number of moderate injury and moderate-severe injury animals with a long or short duration. At the late time point, there were significantly fewer moderate injury animals with long durations as compared to moderate-severe injury animals ($p < .01$). There were no differences within injury groups between time points (Figure 10B).

PAS was determined by the distance traveled over time by the penile body from rest to the point of MAPD. Median split was used to determine high (> 7.7 cm/s) versus low (≤ 7.7 cm/s) speed. The moderate injury group had significantly fewer animals with high speeds at early time point as compared to the moderate-severe injury group ($p < .05$). At the late time point, the number of moderate injury animals presenting with high PAS was significantly fewer than that of moderate-severe injury animals ($p <.05$). There were no differences within injury groups between the early and late time points (Figure 10C).

MAPD and TPED are inversely correlated with WMS

MAPD and TPED were compared against percent WMS using the Spearman Rank Correlation test. MAPD was inversely correlated with percent WMS ($\rho = -.507$), where with increasing WMS there were smaller MAPD (Figure 11A). Similarly, TPED was inversely correlated with percent WMS ($\rho = -.410$), where with increasing WMS there were shorter durations of the TPED (Figure 11B).

Discussion

During the PDFR, penile glans cupping and penile dorsiflexion are directly related to copulatory behavior, where glans cupping ensures proper seminal plug placement against the female's cervix and penile dorsiflexion allows for intromission[157, 187, 222]. The PDFR is difficult to elicit in the intact rat as it is under tonic descending brainstem inhibition. If the PDFR response does occur in the intact rat, the penile dorsiflexions and glans cupping are less extreme as what is seen in the spinally lesioned rat[150, 152]. Supra-spinal tonic bilateral descending inhibition originates in the nucleus paragigantocellularis (nPGl) of the medullary reticular formation, and travels through the reticulospinal pathway within the lateral funiculus in the rat[222]where these projections eventually reach the motoneuron pools in the lumbosacral cord controlling the perineal musculature, specifically the bulbospongiosus (L5-L6) and the ischiocavernosus (L6-S1) muscles[26, 59, 122]. Disruption of these descending projections allow for a hypersensitivity of the reflex circuitry responsible for the PDFR. In this study, moderate injury animals with a percent WMS between 20.85-33.5% had significantly fewer instances of PDFR than moderate-severe injury animals (WMS 13.33-17.15%) at both early and late time points. This difference is consistent with the medio-lateral and dorso-ventral distribution of reticulospinal fibers[222] whereby a bilateral contusion injury with a central core lesioned area and differential rim sparing infringes to different degrees upon the lateral funiculus (see Figure 7 examples).

Intensity scoring, where glans engorgement and penile dorsiflexion were qualitatively categorized based upon cup intensity and penile angle observation, has previously been used as a method of scoring the PDFR [26, 157, 192]. In this study,

2D kinematic analysis was used as a method to gather a more robust quantification of PDFR intensity. The moderate injury group had significantly fewer animals with large MAPD at both early and late time points, suggesting overall less intense penile dorsiflexion. The duration of the penile dorsiflexion from first movement from rest, to maximum angle, back to rest demonstrates the sensitivity of the perineal musculature motoneuron pool. In the current study, the moderate injury group had significantly fewer animals with long event durations at the late (more chronic post-SCI) time point. As TPED is defined as the duration from the first penile movement from rest to the return of the penile body to rest, TPED measurement includes the duration at which the penis remains at the maximum angle reached. During PDFR, pulsatile contractions of both the BSM and ICM allow for “elongated” dorsiflexions to occur, which is atypical of copulatory behavior[186, 223]. Therefore, long PDFR duration suggests a more severe sexual deficit phenotype which is more prominently seen in the moderate-severe injury group and consistent with a larger injury extent. Greater intensities of the PDFR as measured by MAPD and TPED in the moderate-severe injury group is likely due to increased hypersensitivity of the perineal motoneurons, where the ICM and BSM of moderate-severe injury animals have an increased contraction in response to initiation of the PDFR as compared to that of the moderate injury animals.

Penile dorsiflexion speed has previously yet to be examined as a measure of sensitivity in the PDFR. The PAS is the measurement of distance (cm) over time (s) with which the penile body moves towards MAPD or alternatively, PAS is representative of pulsatile contractions of the ICM[186]. Penile dorsiflexion speed

measurements are yet another measure of intensity, though examining the speed with which the ICM can propel penile dorsiflexion allows an additional examination of ICM motoneuron hypersensitivity after SCI. Here, we see that numbers of moderate injury animals with high PAS is significantly fewer than that of moderate-severe injury animals at both early and late time points, suggesting an increased hypersensitivity of the ICM motoneurons in the moderate –severe injury group. Though we expected to see differences between injury severity groups at both time points in all measured parameters, PDFR duration differences were only seen at late time points. This is likely due to recovery that is still ongoing at early time points and is likely complete by late time points[224].

The reticulospinal tract travels through the dorsolateral quadrant of the rat spinal cord at the level of injury in this study (T9), and therefore receives significant insult with contusion injury[222]. Overall, the differences between intensity measurements in the PDFR between injury severity groups is likely due to the residual reticulospinal projections traversing the lesion epicenter and remaining reticulospinal pathways in the rim of the dorsolateral cord, where more percent WMS is indicative of increased spared descending bilateral tonic inhibition onto the circuitry responsible for sexual reflexes. Indeed, both MAPD and TPED are inversely correlated with WMS, where increases in percent WMS led to decreases in angle size and duration respectively, supporting the idea for a discreet pathway for supraspinal control over the coordination of perineal musculature. Utilizing 2D kinematic analysis of the PDFR allows for a more consistent and quantitative measure of PDFR intensity, allowing for detection of more subtle differences

occurring between injury severity groups than observational approaches. In addition, the added methodological precision of 2D kinematics for sexual function provides a quantitative outcome measure for studies that test therapeutic interventions in clinically relevant incomplete contusion rodent models of SCI.

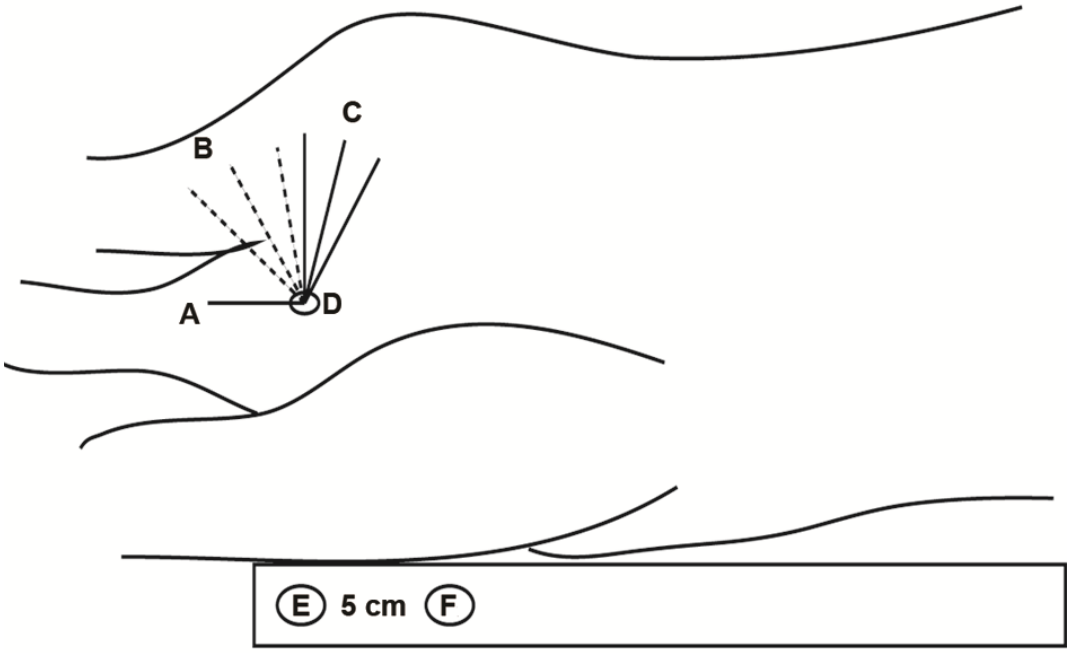

Figure 6 Schematic of PDFR kinematic recording set-up.

Line A represents the penis at rest after prepuce retraction. Lines B (dashed) are representative maximum angles of penile dorsiflexion of animals with a moderate injury. Lines C (solid) is maximum angles of penile dorsiflexion of animals with a moderate-severe injury. Point D is representative of the marker placed on the base of the penis during PDFR testing. Points E and F are representative of the permanent reference markers on the PDFR testing platform. The angle between the vectors created by points E-F and A/B/C-D is the measured angle for MAPD. The permanent markers were placed 5 cm apart to allow for distance calibration. This schematic is not drawn to scale. MAPD – Maximum Angle of Penile Dorsiflexion; PDFR – Penile DorsiFlexion Reflex.

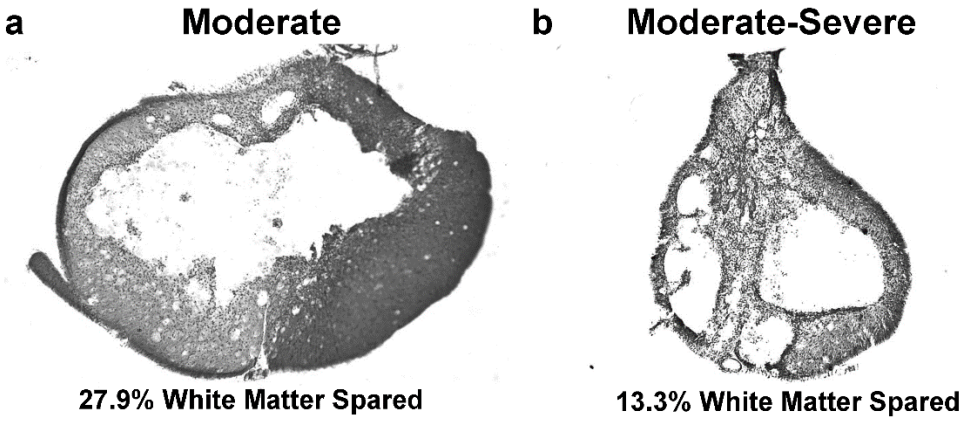

Figure 7 Representative spinal cord lesions.

Representative spinal cord segments at the lesion epicenter of a moderate injury in "a" above 20% white matter sparing (actual value range for current study animals: 20.85-33.5% WMS) and a moderate-severe injury in "b" above what would be considered more severe (between 0 and 5-10% sparing) but below 20% (actual value range for current study animals: 13.33-17.15% WMS) SCI animal.

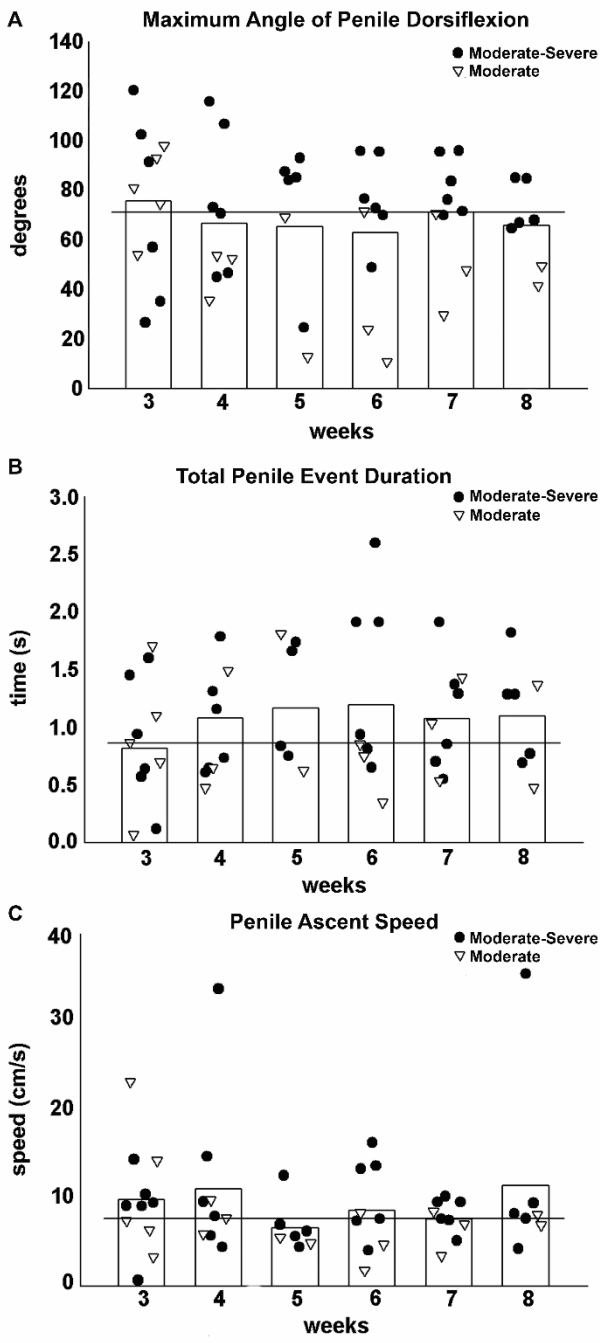

Figure 8 Descriptive data of PDFR.

Distribution of individual rat data over six weekly testing periods (weeks 3 to 8 post-SCI). (A) Maximum Angle of Penile Dorsiflexion (MAPD); (B) Total Penile Event Duration; and (C) Penile Ascent Speed. The horizontal line represents the median measurement.

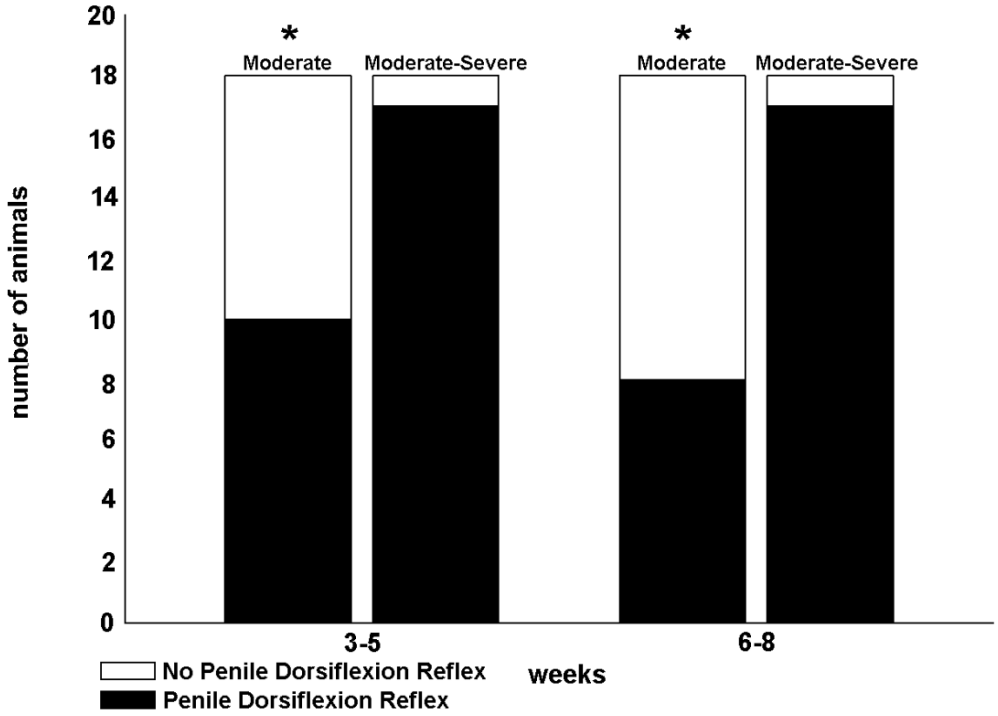

Figure 9 Penile dorsiflexion reflex occurrence in moderate vs moderate-severe injured animals at early and late timepoints.

The number of moderate animals presenting with a positive PDFR test was significantly fewer than those with moderate-severe injuries at the early time point (Weeks 3-5; Mod n=6; Mod-Sev n = 6; $p < .005$). At the late timepoint, the number of moderate animals with a positive PDFR test was significantly fewer than positive PDFR in moderate-severe animals (Weeks 6-8; Mod n=6; Mod-Sev n = 6; $p < .001$).

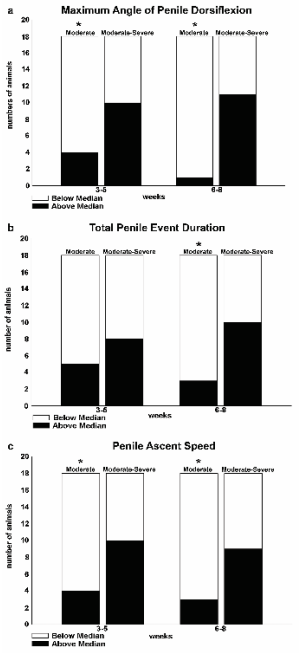

Figure 10 Injury severity affects Maximum Angle of Penile Dorsiflexion, Total Penile Event Duration, and Penile Ascent Speed high and low performers at early and late timepoints.

A) The number of moderate injury animals to have a large maximum angle of penile dorsiflexion was significantly fewer than the number of moderate-severe injury animals to have a large maximum angle of penile dorsiflexion at both early (Mod n=6; Mod-Sev n = 6; $p < .05$) and late (Mod n=6; Mod-Sev n = 6; $p < .001$) time points. B) The number of moderate injury animals with a long total penile event duration was significantly fewer than that of the moderate-severe injury animals at late time points (Mod n=6; Mod-Sev n = 6; $p < .005$). C) The number of moderate injury animals to have a high speed of penile ascent was significantly fewer than the number of moderate-severe injury animals to have a high speed of penile ascent at

both early (Mod n=6; Mod-Sev n = 6; $p < .05$) and late (Mod n=6; Mod-Sev n = 6; $p < .05$) time points.

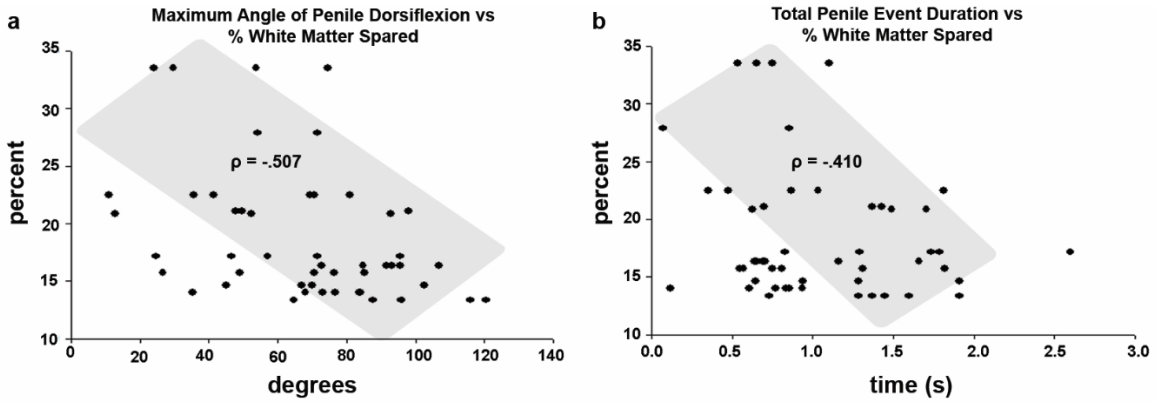

Figure 11 Percent white matter spared versus Maximum Angle of Penile Dorsiflexion and Total Penile Event Duration.

A) Maximum angle of penile dorsiflexion across all six weeks of analysis is inversely correlated with the percent of white matter spared (Mod n=6; Mod-Sev n = 6; ρ = -.507). B) Total penile event duration across all six weeks of analysis is inversely correlated with the percent of white matter spared (Mod n=6; Mod-Sev n = 6; ρ = -.410). Shaded area indicates negative trend in correlation, where Spearman's rank coefficiant is represented by ρ.

CHAPTER IV

TELEMETRIC MONITORING OF PENILE PRESSURE DURING MATING IN RATS AFTER CHRONIC SPINAL CORD INJURY

Introduction

Spinal cord injury (SCI) leads to multifunctional disruption of neurological symptoms, including deficits in sexual function. SCI individuals rate sexual dysfunction as a top priority quality of life issue[21]. Typical deficits in sexual function after SCI include erectile and ejaculatory dysfunction, as well as decreased fertility[10]. Erectile dysfunction is the most widely reported cause for dissatisfaction with sexual function amongst SCI males[3], as erections are most often short-lived and lack the rigidity required for penetration, though they may first be easily initiated[6, 8]. Additionally, 95% of SCI males with lesions above T10 report ejaculatory dysfunction, with impairments including perineal musculature dyssynergia resulting in non-forceful expulsion of ejaculate, anejaculation, or retrograde ejaculation due to improper bladder neck closure[49, 50, 54].Despite the high priority of sexual dysfunction amongst SCI individuals, limited studies exploring potential mechanisms have been conducted, with the area of post-SCI animal research receiving the least attention[167].

Historically, longitudinal studies of sexual dysfunction have been limited by availability of quantitative outcome measures. Such methodologies have included intracavernosal (ICP) and intraspongiosal pressure (ISP) recordings in response to cavernous nerve, pelvic plexus, and dorsal nerve of the penis (DNP) electrical stimulation or pharmacologically induced erections[154]. Additionally, mating tests and *ex copula* sexual reflex tests, non-contact erection test [225], and EMG of perineal musculature during mating tests[226, 227] have all been used in measuring of sexual function. Limitations from such studies include non-physiological test settings, use of anesthesia, lack of robust objective quantification (regarding mating tests and *ex copula* sexual reflexes), and single time point of testing. While a wealth of knowledge pertaining to sexual function after SCI has been gained through studies utilizing these methods (see Alexander and Marson 2017 for review[228]), the increasing use of telemetric pressure transducers allows for a refinement of quantitative methods of chronic assessment in physiologically-typical scenarios.

Telemetric recording of the ICP (erectile only) or of the ISP (micturition and erectile) has been successfully used in animal models of sexual function during ex copula sexual reflexes, sleep erections, spontaneous erections, as well as real-time mating behavior tests [123, 157, 207, 210, 229-233].Telemetric ISP monitoring has been utilized in an SCI model assessing *ex copula* sexual reflexes[157]; however, mating behavior in a SCI model has yet to be examined.

In the current study, a clinically relevant chronic SCI animal model implanted with a telemetric pressure transducer was used to determine the effect of SCI on

ICP and mating behavior events including duration, as well as whether a potential relationship exists between extent of injury severity and emerging sexual deficits.

Methods

Animal Care

Protocols were reviewed and approved by the Institute Animal Use and Care Committee at the University of Louisville, School of Medicine (# 14025), and all procedures were carried out to the National Institutes of Health guidelines. Sixteen adult male Wistar rats (~300g) and eight adult female Wistar rats (~200g) were individually housed on a standard 12-hour light/dark cycle. Male rats were first implanted with telemetry devices, had baseline behavioral assessments, and then were randomized to either one of three T9 level contusion severity groups to insure a range of incomplete injury extents (150, 175 or 215 kilodyne injury forces; n=4/group) or to a sham laminectomy surgical control group (n=4). All SCI's were performed with the Infinite Horizons Impactor (Precision Systems and Instrumentation, LLC; Fairfax Station, VA) (47). Overground locomotion was assessed at pre-operative and post-operative (once a week post-SCI) time points using the Basso, Beattie, and Bresnahan (BBB) scale[179]. The mating behavior paradigm using a sexually receptive female (see below) was performed 2-5 times pre-injury (including handling) and at weekly intervals beginning at post-injury week 3. A time-line for device implantation, SCI, and behavioral testing is provided in Figure 12.

Surgeries

Pre-SCI Telemetric Pressure Transducer Implantation. Male rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg, Ketoset ®; Fort

Dodge Laboratories, Fort Dodge, IA) and xylazine (10 mg/kg, AnaSed; Lloyd Laboratories, Shenandoah, IA). After surgical preparation, animals were placed in a dorsal recumbency and received a rostral/caudal midline incision (3 cm) in the perineal tissue to expose the bulbospongiosus muscle (BSM). The animals were then turned to a prone placement, and a 3 cm subcutaneous incision was made directly above the right hip. Blunt dissection was used to create a tunnel from hip to perineum, through which the telemetric pressure transducer catheter (TA11PA-C40; Data Sciences International, St. Paul, MN) was threaded to reach the base of the penis. The pressure catheter battery/transducer was secure with two 4-0 Ethicon non-absorbable sutures to the subcutaneous fascia, and the skin closed with Michel clips. The animal was then returned to a dorsal recumbency, where the base of the penis was exposed via a minimal amount of BSM dissection. Using a 21-gauge needle, a small guide hole was made to allow the open tip of the catheter to be placed in the right proximal shaft of the corpus cavernosum. The pressure catheter was gently secured with two 4-0 Ethicon non-absorbable sutures on the BSM, and all openings were securely closed with 4-0 Ethicon non-absorbable surgical suture and Michel clips for skin closure. Animals were given a full week of recovery prior to behavioral testing.

Female ovariectomy. Two female Wistar rats were used for every four males. Female rats (n=8) were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) for ovariectomy using previous lab protocols [102, 234]. The surgical field was prepared, and a rostral-caudal midline incision was made through which the muscles over the ovarian fat pads were visualized. The

muscle was gently separated, and the fat pad exposed. The oviduct was then ligated, and the ovary removed from the tube. The remaining fat pad and oviduct were placed back inside the body, and the procedure was repeated for the remaining ovary. The skin was then closed with Michel clips. Animals then had a subcutaneous 60-day time-released 17β-estradiol pellet implanted (Innovative Research of America, Sarasota, FL) per our previous studies[102, 235]. Ovariectomies were performed at the time of the male telemetric pressure transducer implantation, and therefore, the females were also given 7 days of recovery prior to being placed in the mating behavior paradigm.

Spinal Cord Injury. The following procedures can be viewed in a video journal recently published by our laboratory[175]. All male animals were surgically prepared and anesthetized using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). After surgical preparation, each animal was subcutaneously injected with sterile saline solution (5 ml) and placed in a prone position upon a heating pad to ensure a maintained 36-37ºC body temperature. A rostral-caudal midline incision exposed the T7-T9 vertebra, the T8 lamina was removed to expose the T9 spinal cord, and the spinal column stabilized at the T7-9 spinal processes with spinal clamps. For the injury animal groups, incomplete lesions were made using the Infinite Horizons impactor with forces pre-determined at either 150 kilodynes (n=4), 175 kilodynes (n=4), or 215 kilodynes (n=4). Sham surgical controls (n=4) did not receive an injury. The muscle and subcutaneous tissue were then closed for all groups using 4-0 Ethicon non-absorbable surgical suture and skin was closed with Michel clips.

Post-surgical care. Immediately after closure, bacitracin (topical antibiotic) was placed on the wound, and all animals received 5 ml of subcutaneous saline, as well as 0.1 ml of Penject ® dual penicillin (The Butler Company, Columbus, OH) as a general prophylactic. In the case of SCI surgery, 0.3 ml of gentamicin (GentaFuse ®; Butler Schein, Dublin, OH) was given immediately post-surgery and continued for 5 days to prevent bladder infection. Meloxicam (0.2 ml; Eloxiject; Henry Schein, Melville, NY) was given twice daily for 3 days post-surgery (in all instances of surgery) for pain management. After SCI, animals' bladders were emptied three times/day using the crede procedure per established protocols[168, 169, 175, 177, 178] until the animals were reflexively voiding without assistance. After telemetric pressure transducer catheter placement, animals were given one-week recovery prior to any behavioral testing. Animals were also given two-weeks recovery time prior to behavioral testing after SCI.

Mating Behavior Paradigm

Pre-Injury mating behavior testing began 7 days after telemetric pressure transducer placement and continued until each animal was no longer considered sexually naïve (three ejaculations[155]). Mating behavior testing occurred during the 12hr dark cycle, beginning at 1900 hrs. Ovariectomized female rats were hormonally controlled using a 60-day estradiol implant and induced for receptivity with progesterone injections at 24-hr and 6-hrs prior to mating behavior testing[155]. Prior to mating behavior testing, pre-estrus status was verified using a vaginal lavage and cell examination [236]. Animals were first habituated to the testing environment for

10 minutes to allow time for exploration of the behavioral unit (clear polypropylene breeding cage; 18.25 in L x 12 in W x 6.25 in H), during which baseline ICM pressures were recorded. One pre-estrus-induced ovariectomized female rat was then placed in the behavior unit with the male. The male rat remained with the female for a total of 30 minutes, or until two ejaculations had occurred, whichever was fulfilled first. Sexual performance was scored by a trained observer, with numbers of mounts, intromissions, and ejaculations recorded. Ejaculation was confirmed by the presence of a seminal plug. Throughout mating behavior testing, Ponemah software (Data Sciences International, St. Paul, MN) was used to record the ICP. Post-SCI testing resumed after a 14-day recovery period (beyond the period of spinal shock [178]) and was performed weekly during post-injury weeks 3-8 (Figure 12).

Mating Behavior Analysis

During mating behavior testing, mounts, intromissions, and ejaculations were marked with a cursor in the Ponemah program. Ponemah ICP recordings were exported to LabChart (ADInstruments, Colorado Springs, CO), where events were marked with cursors corresponding to the beginning and end of each mating event (mount, intromission, ejaculation). The first cursor was placed where the pressure first departed from baseline pressure and the final cursor placed where the pressure returned to baseline. LabChart files were then exported into Excel (Microsoft Office, Seattle, WA), where software written in Visual Basic for Applications (VBA Excel) specifically for this analysis confirmed the numbers of behavior events observed

during testing, as well as determined the average pressure and duration of mounts, intromissions, and ejaculations.

Histology of lesion epicenter

After the final mating behavior testing, animals were lethally dosed with anesthesia and perfused with a paraformaldehyde/heparin solution and the tissue processed as previously described (24). Post-perfusion, the T6-T12 spinal cord was removed and post-fixed in a 4% paraformaldehyde solution for 2-4 days at 4ºC. The tissue was placed in a 30% sucrose/phosphate buffer solution 24-hours prior to sectioning. The tissue was transversely sectioned at 20µm thickness on a cryostat, slide-mounted, and stained using the Kluver-Barrera method to visualize the white and gray matter.

Using a Nikon E400 microscope and Spot Advanced software (Diagnostic Instruments, Sterline Heights, MI), tissue images were captured for analysis and measurements were obtained to quantify lesion extent. Total white matter damage/sparing was determined based on averaged intact areas from above and below the level of injury at the lesion epicenter, as previously described [177, 182]. The dorsal columns, dorsolateral funiculus, ventrolateral funiculus, and ventromedial funiculus were further assessed as sub-regions. The medial edges of the dorsal horn, ventral horn tips, and the central canal were used as landmarks to determine cord divisions.

Statistical Analysis

Analysis was performed using SigmaStat (Systat Software, INC, San Jose, CA). The Shapiro-Wilk test was performed for normality, and the Brown-Forsythe test for inequality was used to determine equality of variance. Pre-injury pressure analysis was performed using a one-way ANOVA on Ranks. Mating behavior tests were analyzed with repeated measures (ANOVA) (fixed effects) to compare between treatment groups within subjects and between testing time-points. The Holm-Sidak post hoc t-test was used as the multiple comparison test. Pearson correlations were used for all tests versus bilateral percent white matter sparing. All values reported are ± standard error of means (SEM) unless otherwise noted.

Results

This study utilized telemetric pressure transducers to examine the ICP in awake, behaving animals. The number of mounts, intromissions, and ejaculations were recorded during each mating session by a trained observer, where each event was marked with the appropriate event marker in the Ponemah program. Ejaculations were confirmed with visualization of a vaginally deposited seminal plug. ICP was recorded by the device throughout the testing session.

As a range of injury severities were attempted and variability can arise even under rigorous conditions, analysis was done for the actual force and displacement values for each animal recorded from Infinite Horizon generated software program as well as white matter sparing data from histological tissue measurements. For white matter sparing (WMS), two distributions split 50% above and below the mean with significant differences between the means of those two groups ($p<0.005$) was found. In contrast, the data for force and displacement were equally distributed regardless of severity. Thus, analysis of the mating data looking for potential differences across time relative to surgical sham controls were done with the SCI animal data as two injury severity subsets (n=6 per group), whose distribution was above and below 20% WMS.

Prior to SCI, for sexual acclimation, the animals were mated with receptive ovariectomized females in 30-minute sessions/day until the male had a cumulative three ejaculations, which resulted in an unequal number of mating sessions prior to SCI (range of two to five sessions). However, post hoc comparisons between shams and injury severity groups with above and below 20% white matter sparing revealed

no significant group differences in mean number of pre-injury mating sessions needed to reach sexual acclimation (average sessions for sexual acclimation - sham: 2.75; above 20% WMS: 3.33; below 20% WMS: 3). Also, based upon pre-injury data from the final mating session where sexual acclimation was reached, post hoc analysis revealed no statistical differences present between sham and injury severity animals in numbers of mounts, intromissions, or ejaculations, confirmation of effective randomization at the time of SCI. There were also no statistically significant differences in ICP pre-injury between the later determined groups. Note that pre-injury mount ICP (n=16) was significantly lower than ICP of both intromissions and ejaculations ($p < .001$; Figure 13), a finding consistent with previous studies by other groups [207].

Mating behavior, ICP, and durations of mating behaviors were compared initially using data averaged across all six post-SCI testing sessions (weeks 3-8 post-contusion) against WMS (Figure 14) using the Pearson correlation test to identify potential lesion size/severity relationships given the range of IH contusion forces applied. Numerous positive correlations included numbers of intromissions ($R=.443$, $p<.001$; Figure 14B) and ejaculations ($R=.453$, $p<.00$; Figure 14C), the ICP of both mounts ($R=.411$, $p<.001$; Figure 14D) and ejaculations ($R=.428$, $p<.001$; Figure 14F), and the duration of ejaculations ($R=.36$, $p<.001$; Figure 14I). Two clusters of data were apparent from the plotted values (identified with open circles and triangles in Figure 14); one cluster above 20% WMS and one below 20% WMS. Per above with respect to the two distributions of WMS split 50% above and below

the mean, further analysis was done with the SCI animal data as two injury severity subsets (n=6 per group); above and below 20% WMS.

Weekly mating behavior analysis began during the third week post-injury after a two-week recovery period, and continued for six weeks, ending at post-injury week 8. Although the number of mounts occurring post-injury, as well as for shams, was unchanged across all weeks as compared to pre-injury numbers (Figure 15A; $p>0.05$), the number of intromissions ($F=12.1$, $df=6,4013.8$, $p<.001$) and ejaculations ($F=9.1$, $df=6,12.1$, $p<.001$) was significantly less after SCI as compared to pre-injury levels at all weeks regardless of percent WMS. In contrast, there was a significant change in ICP post-injury during mount events compared to pre-injury ($F=2.4$, $df=6$, 6634.9, $p=.037$), where post-hoc analysis revealed significantly lower ICP in only the group with less than 20% WMS at all weeks ($p<.001$) (Figure 16A). ICP during intromissions was also significantly changed after SCI ($F=4.65$, $df=6,32146.3$, $p<.001$), where the injury with more WMS had a significantly lower ICP at some weeks (4, 5, and 7; $p<.001$) versus the group with greater extent of damage at the lesion epicenter having significantly lower ICP at all post-injury weeks ($p<.001$) (Figure 16B). With respect to ICP during ejaculations (for the small number that did occur – <20% WMS: 16.6% at Week 3, 4 & 5; >20% WMS: 16.6% at Week 3, 33.3% at Weeks 4 & 5), there were significant changes from pre-injury levels ($F=9.56$, $df=6,96455.6$, $p<.001$), regardless of extent of WMS (Figure 16C). Representative pressure tracings from a sham laminectomy animal and animals having above and below 20% WMS are provided in Figure 17.

Duration of mating events was also examined. Prior to group randomization, there were no statistically significant differences seen between injury groups in duration of any mating event. There were also no significant differences in duration of mount events post-injury or between groups (Figure 18A). Post-injury duration of intromissions was significantly altered ($F=2.62$, $df=6,12.8$, $p=.024$) and was significantly shorter at all post-injury weeks for only the group of rats with less than 20% WMS ($p<.001$) (Figure 18B). Duration of ejaculations (again for the few that were present) was also significantly altered post-injury compared to pre-injury in main effect ($F=2.82$, $df=6,19.5$, $p<.001$) as well as for most weeks regardless of injury severity group (Figure 18C).

It is important to note that post-hoc examination of BBB locomotor score data revealed no significant differences between the above/below 20% WMS (Figure 19), which could reflect differences based upon the mediolateral and/or dorsoventral location of the mating versus locomotor axonal projections within the white matter at the T9 spinal level. Note that the number of mounts occurring post-injury was unchanged as compared to pre-injury numbers (Figure 15A) despite reduced locomotor function.

Discussion

The current study was designed to determine the effects of SCI on mating behavior events (mounts, intromissions, ejaculations) and event duration as well as physiological parameters (i.e., pressure levels) during such events. Pressure measurements of the corpus cavernosum and corpus spongiosum tissues (ICP or ISP) with telemetry devices as a measure of sexual function has been used successfully, adding to the wealth of knowledge pertaining to sexual circuitries [123, 157, 207, 210, 229-233]. Copulatory behavior has been shown to not be altered after device implantation [207]. As the catheter tip is a foreign body placed internally [154], each animal in the current study was observed for fibrotic reaction upon sacrifice. Even with long-term (total of 10 weeks) placement, animals had little to no penile scarring near the catheter tip. Furthermore, there were no significant changes in pressure readings in any animal group in post-injury weeks, supporting lack of fibrotic influence on pressure recordings. In addition, such erectile pressure monitoring has been implemented for examining *ex copula* sexual reflexes and 24-hr spontaneous erectile events in a contusion SCI model at 7- and 21-days post-SCI [157].

Copulatory behavior in rats is traditionally scored in three components: mounts, intromissions, and ejaculations[155]. Mounts are performed from the rear with the forepaws on the haunches of the female with no vaginal penetration. Intromission is when the penis first enters the vaginal canal, and ejaculation is the expulsion of seminal fluid, which is behaviorally accompanied by a longer duration of intromission and raising of the forepaws[155]. In the present study, a small increase

in ICP occurred during mounting, which was significantly lower than what was measured during intromissions and ejaculations, an outcome consistent with previous studies[207]. In this study, numbers of mounts after SCI injury did not change between weeks or between groups, despite motor deficits, showing that SCI animals were still motivated to seek mating with a receptive female. Additionally, mounts were unchanged in duration relative to pre-injury mount durations. As tumescence does occur during mounts, unchanged duration of pressure above baseline during mounts after injury suggests SCI animals are still capable of achieving the vascular filling component of erectile function during mounting behavior. Similarly, SCI human males are capable of a vascular response to arousal, where such response may be due to genital stimulation (reflexive erection) or mental arousal (psychogenic erection)[10, 11]. The ability of such erections is dependent upon the level of the spinal lesion, where reflexive erections require an intact sacral cord, and psychogenic erections require an intact thoracolumbar sympathetic pathway[10-12, 167]. Though such erections may be elicited, men with SCI report that they are short-lived and lack the rigidity required for penetration[8].

Pressures in animals with below 20% of WMS were significantly lower than pre-injury mount pressures, suggesting that parasympathetic control of penile vascular filling during erection may be compromised with a more severe injury. Indeed, pressure during mounting events was found to be positively correlated with percent of white matter spared across both injury groups. After SCI in humans, one study of men with ‘complete’ SCI reported 61.8% had unreliable erections, with only 17.1% of these men rating erections as “very firm” [8]. Disruptions of NOS mediated

vascular filling through the pudendal nerve[22, 237, 238] may limit cavernosal blood flow during erections of SCI men; though lack of, or limited, perineal muscular contraction likely does not allow elicited erections to reach full necessary rigidity as well.

Mounting shifts to intromission when genital sensory information of perineal female contact allows for an increase in penile rigidity and dorsiflexion of the penis to permit vaginal insertion[149]. All SCI rats had a significant decrease in the number of intromissions as compared to pre-injury values, reflecting insufficient rigidity and impaired penile dorsiflexion necessary for insertion[149]. This impairment is again like what is seen in human SCI who rate a significant portion of their penile responses as unreliable for proper sexual function[8]. Noteworthy in the present study is that the number of intromissions was positively coordinated with percent white matter spared. When intromissions did occur, SCI animals had significantly lower pressures at post-injury weeks, while only animals with less than 20% WMS had significantly lower duration of intromissions. Multiple factors likely contribute to the decreased pressures and durations of intromissions after SCI. Autonomic pro-erectile outflow (vascular filling) occurs first during mounting, while somatic inflow causing perineal musculature activation occurs second and allows for intromission to occur [22]. Thus, for the instances where SCI rats were able to achieve intromission, the shorter durations and/or lower pressures could be explained by a lack of coordination between the autonomic and somatic circuitries. Prior to injury and in sham animals, these mechanisms are very coordinated, with a sharp increase in pressure during intromissions, a length of time spent at peak pressures, and a sharp

decrease back to baseline indicating sufficient ICM and BSM pulsatile contraction allowing for both rigidity and necessary intromissive angle[210]. Typically, these rapid shifts from rigid erection to flaccid state seen in rat copulatory behavior is indicative of highly coordinated parasympathetic and sympathetic sexual neural circuitries. After SCI, extended periods of peaks and valleys with low pressure indicates discoordination and overall weakened perineal muscles, causing increased ICP to be unreachable based on fluid mechanics, in conjunction with a dis-coordinated autonomic influence. Additionally, afferent information is critical for reinforcement of perineal musculature activation, and therefore, decreased sensation during copulation may not allow the necessary input for proper contraction of the ICM for a successful rigidity phase[22].

The results showing differences in duration of intromissions in animals clustered below but not above 20% WMS suggest a role of residual connections traversing the lesion site; however, the expected correlation between pressure or duration of intromission and percent white matter spared is not seen in the current data. Differences of erectile function amongst SCI men is dependent on lesion level and severity. Complete and incomplete upper motor neuron injuries have a higher occurrence of erection (reported 99% and 93%, respectively); however, reported erections were only of sufficient rigidity and duration for penetration in 53% and 63% of cases, respectively[239]. Erectile capabilities in SCI men with complete (26%) and incomplete (90%) lower motor neuron injuries also had limited sufficiency and longevity for penetration at 23% and 80% respectively [239]. Therefore, descending and ascending intact spinal tracts seemingly alter erectile function in SCI males.

For ejaculation, where seminal fluid is forcefully expelled and is behaviorally concomitant with a delayed dismount and a raising of the forepaws, there is a refractory period of 4-10 minutes in which the female may be checked for a seminal plug to confirm ejaculation[155]. Ejaculatory behavior after SCI had the most robust differences as compared to pre-injury, where the numbers of ejaculations were significantly fewer in all SCI rats regardless of degree of WMS. In humans, approximately 95% of SCI males with lesions caudal to T10 have ejaculation impairment [36-38, 71]. Specifically, damage to either the sympathetic outflow arising from the T10-L2 cord (responsible for seminal emission and bladder neck closure), sacral control of contraction of the perineal musculature, (responsible for expulsion), and/or loss of descending connections to either of these circuitries can lead to anejaulation, retrograde ejaculation, or dribbling ejaculation[71]. SCI likely compromises the integrity of the autonomic coordination of the ejaculatory reflex, in addition to disrupting input from pro-ejaculatory supraspinal centers[195]. In the few times that ejaculations did occur in the injured animals, both the duration and the pressure were significantly decreased as compared to pre-injury levels and both were positively correlated with percent of WMS. These deficits in ejaculatory function are likely due to lack of strength and coordination of the BSM, in addition to discoordination of the somatic and sympathetic sexual circuitries responsible for proper ejaculation. BSM removal has been shown to negatively impact ejaculation, as the cup necessary for proper seminal plug placement is not able to be formed, and males without BSM likely retract part of the seminal plug upon dismount[187]. All suspected ejaculations were confirmed with the presence of a seminal plug;

however, worth noting is that at least on one occasion there was residual seminal plug retained in the prepuce of the mating male. Future studies using this model of sexual dysfunction after SCI should examine retained seminal plug and possible retrograde ejaculation into the bladder. Retrograde ejaculation in SCI males occurs due to neurogenic bladder dysfunction, specifically improper bladder neck closure during seminal emission[49, 50]. Examination for possible retrograde ejaculation or retained seminal plug in the cases where an ejaculatory pattern of behavior occurred, but could not be confirmed with seminal plug presence in the female vaginal canal may further elucidate the effects of autonomic-somatic discoordination on ejaculatory function after SCI.

Existing correlations with multiple parameters and white matter sparing and instances of differences between animals clustered by WMS above and below 20% implicates the residual fibers traversing the injury playing a role in the sexual capabilities after SCI. Alterations and damage to ascending sensory fibers[153] and descending supraspinal control of the sexual circuitries, both inhibitory[23, 26, 59, 122, 183-185] and excitatory[12, 149, 240], allow for discoordination of the autonomic (parasympthatheic and sympathetic) and somatic circuitries responsible for proper sexual function after SCI. The reticulospinal pathway, conveying both descending and ascending information to and from supraspinal centers of sexual integration, projects through the dorsolateral quadrant of the spinal cord at the T9 level (level of injury in this study), a region highly impacted with the contusion forces/displacement impactor forces used in the current experiment. Disruption of sexual function correlated with WMS as seen in this study is consistent with the

medio-lateral and dorso-ventral distribution of the reticulospinal fibers[222] where differential rim sparing with varying degrees of lateral funiculus compromise likely allows the less severe functional outcome measures shown with increased WMS.

Conclusion

Overall, this study using telemetry measures reveals deficits in mating behavior events and duration, as well as ICP after SCI. Disruption of the descending supraspinal influences and ascending sensory information, as well as parasympathetic and sympathetic discoordination likely contribute to the mechanisms underlying the various aspects contributing to sexual dysfunction after SCI, from arousal to ejaculation. The use of telemetric pressure monitoring in awake, behaving animal models of SCI is feasible, allowing for robust quantitative measurements of sexual function over chronic timepoints.

EXPERIMENTAL TIMELINE: Telemetric Monitoring of MBT										
	WEEK 0 Pre-injury	Week 1 Pre-Injury		Week 2	Week 3		Week 4	WEEKS 5-9	WEEK 10	
Days	-21 through -15	-14	-13 through -8	-7 through -1	0	1 through 6	7 through 13	14 - 34	35-41	
Animals Arrive	ACC	Telemetric Pressure Catheter Implant	REC	MBT	SCI	REC	REC	Weekly MBT	MBT	TR

Figure 12 Experimental Timeline

Experimental Timeline for Pre-Injury mating testing, SCI, and Post-Injury mating testing and sacrifice. Days shown are relative to day of SCI. ACC - acclimation period handling; MBT – mating behavior testing; REC – recovery; SCI – spinal cord injury; TR – tissue removal.

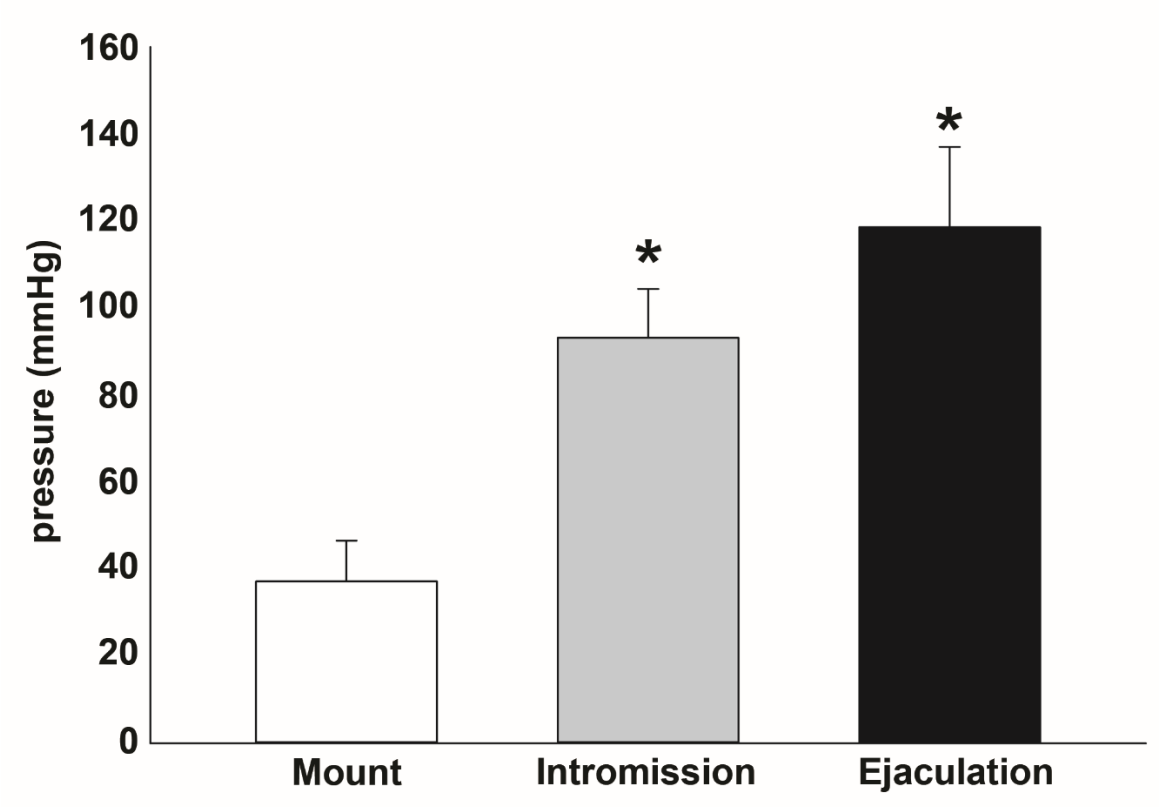

Figure 13 Pre-injury pressures between mounts, intromissions and ejaculations. The mean pressure of intromissions and ejaculations pre-injury for all rats combined were significantly higher than that of mean mount pressure. There was no significant difference between the mean pressure of intromissions vs ejaculations. (* denotes significance relative to mounts; $p<.01$, means ± SEM)

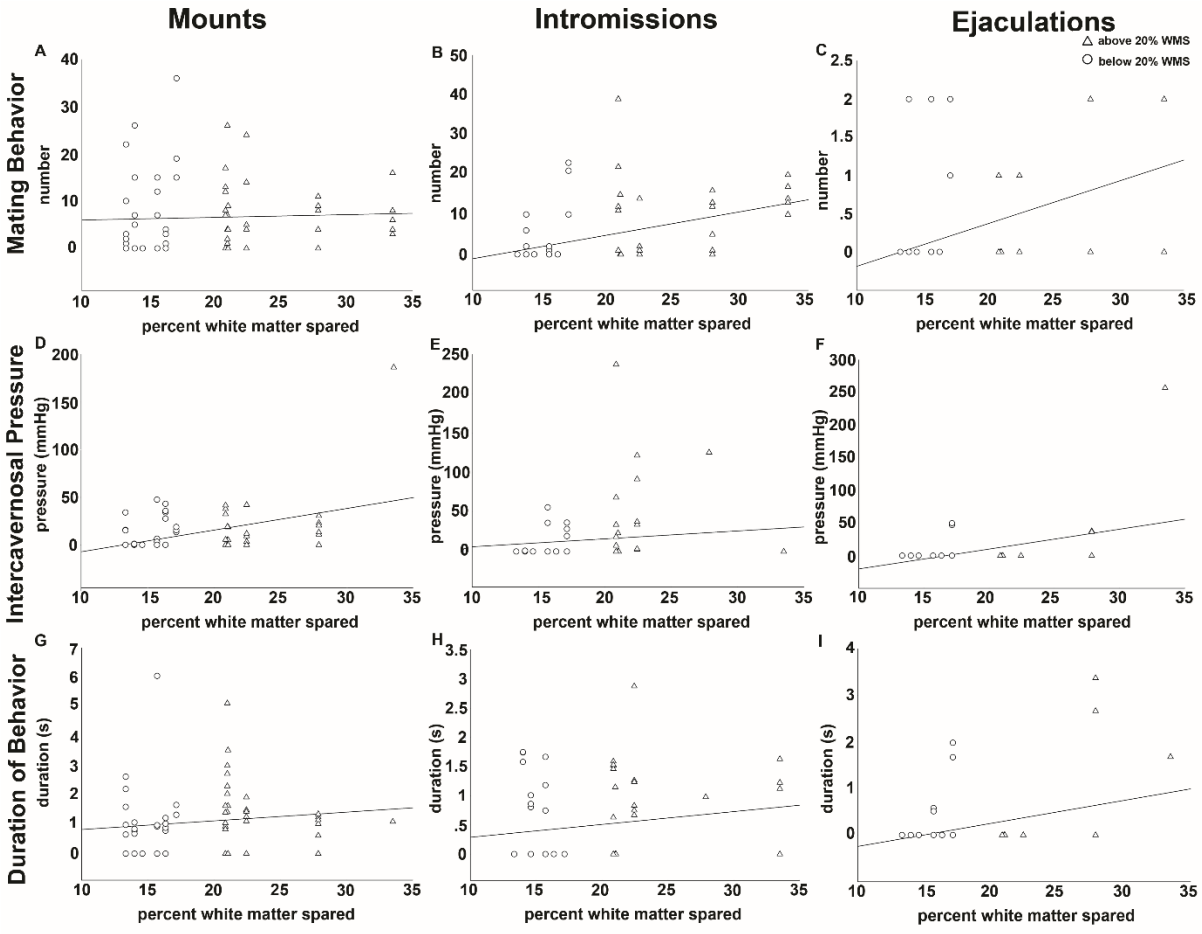

Figure 14 Mating behavior parameters vs percent white matter spared.

There were no correlations between number of mounts and percent white matter spared (A). In contrast, there was a positive correlation between number of intromissions and percent white matter spared (B; r=.443, p<.001), number of ejaculations and percent white matter spared (C; r=.453, p<.001), and mean pressure of mounts and percent white matter spared (D; r=.411, p<.001). There was no correlation between pressure of intromissions/duration of mounts and intromissions relative to percent white matter spared (E, G and H). (F) There was a positive correlation between mean pressure and duration of ejaculations and percent white matter spared (F; r=.428, p<.001 and I; r=.36, p<.001).

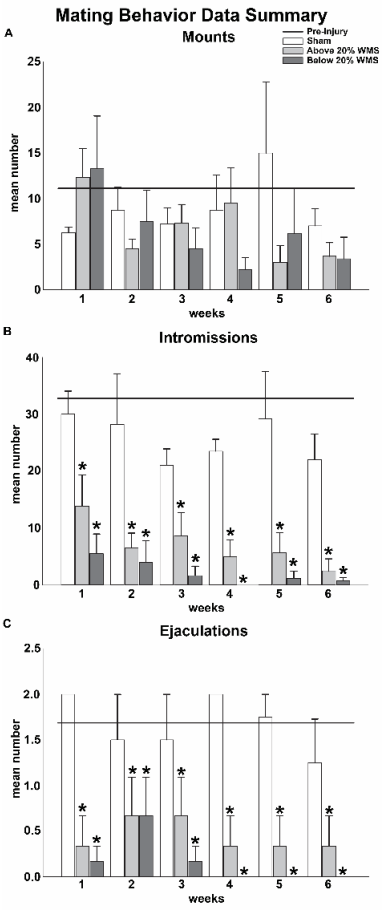

Figure 15 Mating behavior data summary.

The number of mating behavior events of mounts (A), intromissions (B), and ejaculations (C). (A)There were no significant differences of number of mounts at any week compared to pre-injury. (B) The mean number of intromissions of both animals with greater than 20% WMS and less than 20% WMS was significantly lower than that of pre-injury intromissions at all week's post-injury. (C) The mean number of ejaculations of both animals with greater than 20% WMS and less than 20% WMS was significantly lower than that of pre-injury intromissions at all week's post-injury. There were no differences between injury groups. (* denotes p<.001, means ± SEM)

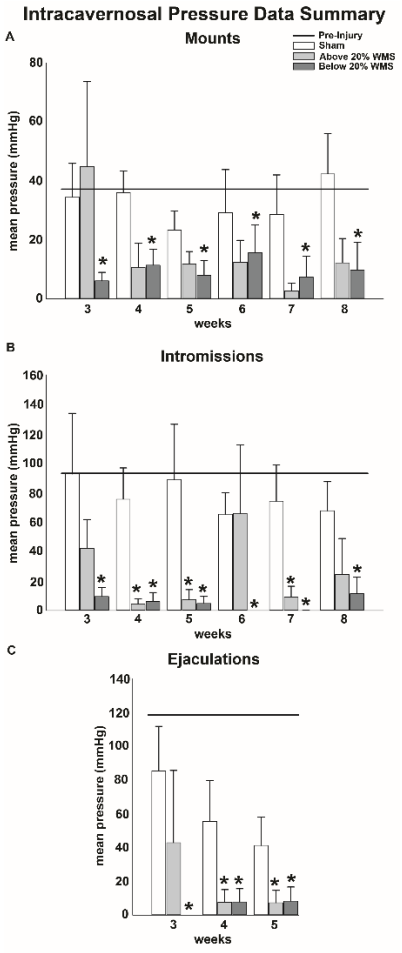

Figure 16 Intracavernosal data summary.

The mean intracavernosal pressure of mating behavior events of mounts (A), intromissions (B), and ejaculations (C). (A)The mean pressure of mounts of the below 20% WMS group only was significantly lower than that of pre-injury intromissions at all weeks. (B) The mean pressure of intromissions of both above (weeks 4,5,7) and below (all weeks) 20% WMS injury groups was significantly lower than that of pre-injury intromissions. (C) The mean pressure of ejaculations of both above (week 4) and below (weeks 3-5)20% WMS injury groups was significantly lower than that of pre-injury intromissions. No ejaculations occurred in injured animals during weeks 6-8. (* denotes $p<.005$, means ± SEM).

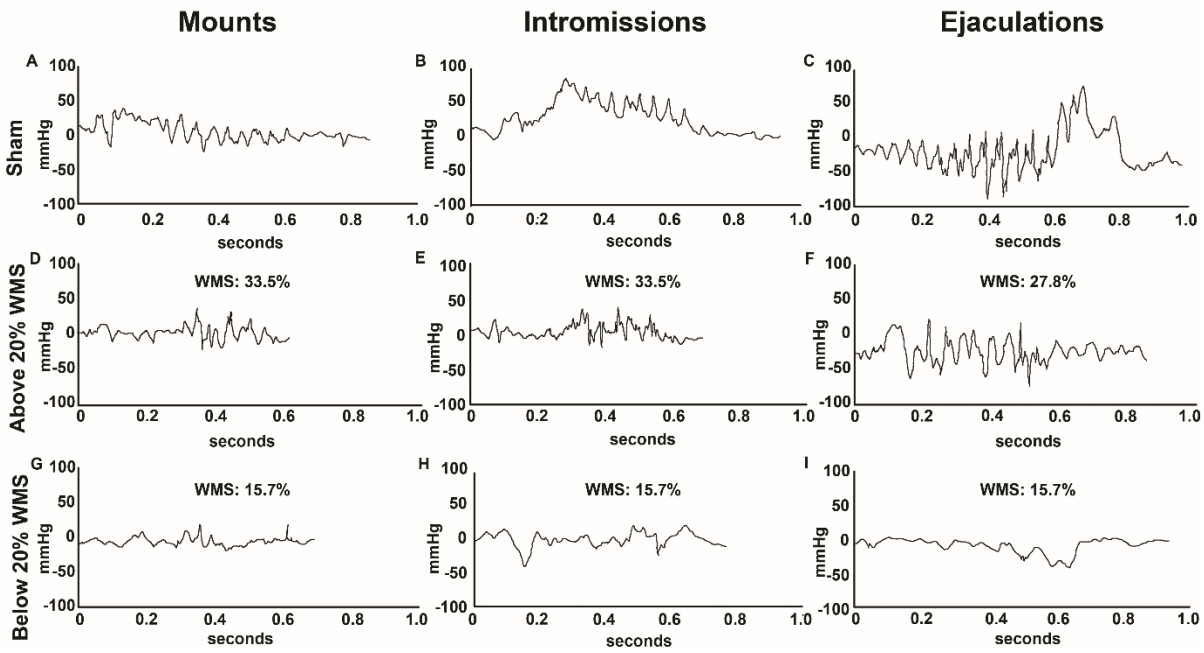

Figure 17 Representative telemetric pressure transducer responses. Responses during mounts, intromissions and ejaculations for surgical sham controls (A-C), above 20% WMS animals (D-F), and below 20% WMS animals (G-I). Note the pressure differences regardless of WMS animals relative to shams for all mating behaviors except for mounts for above 20% WMS.

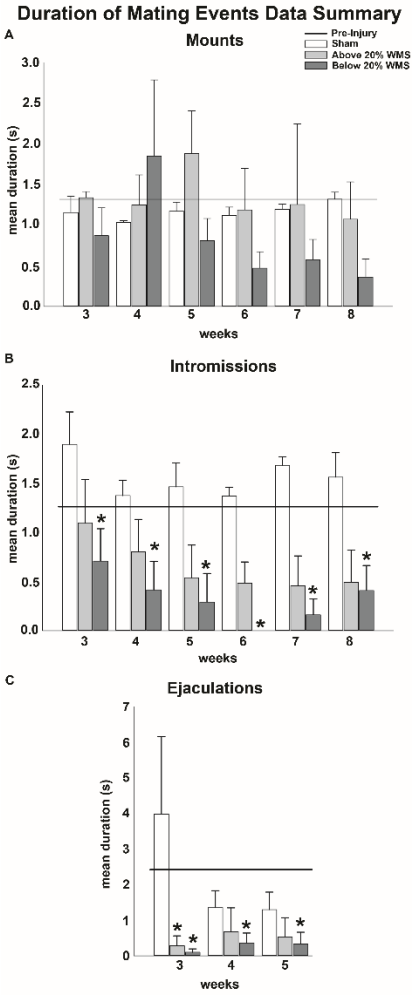

Figure 18 Duration of mating events data summary.

The mean duration of mating behavior events of mounts (A), intromissions (B), and ejaculations (C). (A)There were no differences of mean duration during mounting events as compared to pre-injury. (B) The mean duration of intromissions of the below 20% WMS group was significantly lower than that of pre-injury intromissions at all weeks. (C) The mean duration of ejaculations of both above (week 3) and below (weeks 3-5) 20% WMS groups was significantly lower than that of pre-injury intromissions. No ejaculations occurred in injured animals during weeks 6-8. (* denotes $p<.05$, means ± SEM)

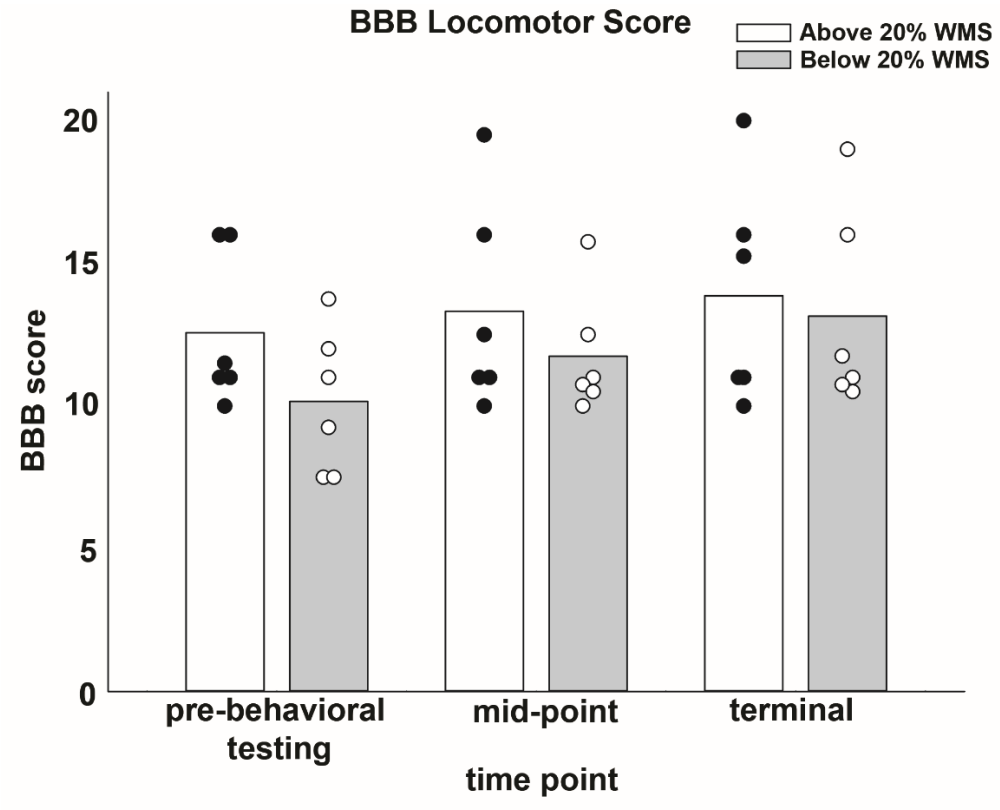

Figure 19 BBB locomotor score.

BBB locomotor scores were averaged between post-injury week 1 and 2 (pre-behavioral testing), weeks 3-5 (mid-point), and weeks 6-8 (terminal). There were no significant differences between injury groups. Although not statistically different, the group having below 20% WMS went from an average BBB score of around 10 (occasional weight supported plantar steps but no forelimb-hindlimb coordination) to 13 (consistent weight supported plantar steps and frequent forelimb-hindlimb coordination), reflecting a post-injury functional improvement of coordination in stepping ability over time.

CHAPTER V

GENERAL DISCUSSION AND FUTURE DIRECTIONS

This project's overall goal was to further investigate using quantitative outcome measures the impact of SCI on male sexual function in a pre-clinical incomplete contusion animal model. Though sexual function is a high priority amongst SCI individuals[21], it remains an under-researched area, especially in regard to animal studies[167, 228]. Overall, sexual function is a highly complex and inter-woven function to study, with several layers of necessary alignment for full function: psychological arousal, penile/clitoral erection, vaginal lubrication, ejaculation, and orgasm. Furthermore, these aspects of full sexual health are dependent on the coordination of multiple different systems: the hypothalamic-pituitary-gonadal hormonal axis, autonomic and somatic nervous systems, as well as the vascular system[12, 46, 241]. Though it is not possible to fully separate these layers and systems, this study seeks to add to the field of knowledge of the neurophysiological aspects of sexual function, and specifically how they are altered after a clinically relevant contusion injury. Additionally, as men are over 80% of the SCI population[21], this study further narrows the focus onto sexual dysfunctions of the SCI male population. However, it should be noted that women with SCI rate sexual dysfunction with similar priority to men[21], and the field of

female sexual dysfunction after SCI has received even less attention by the scientific community[167, 228]. Future studies in both animal and human models regarding sexual function after SCI should share focus with female physiology and intervention.

In the SCI males, it has long been observed that mechanical stimulation of the genital region can elicit a reflexogenic erection, if the sacral cord remains intact[242]. Homologous reflexive penile responses (*ex copula* sexual reflexes) have been found in the rat, dog, rabbit, and mouse[186, 223]. It has since been determined these reflexes are under supraspinal influence from the MRF[26, 59, 122]. Specifically, the PDFR can be elicited in the intact rat[151, 186], as tonic stimulation from the DNP can reduce the inhibition and allow the reflex, consisting of penile dorsiflexions and glans cupping. Complete removal of this descending inhibition allows a more intense PDFR to occur with a much shorter latency[151]. Similar change in intensity of post-SCI erectile responses has not been reported in humans. In fact, though erections are easily achieved, they are often short-lived and lack the rigidity required for penetration[8] [6]. It is possible these easily elicited erections in humans after SCI is due to a disruption of descending inhibition of sexual circuitries as we see in animal models, while the differences in intensity from the animal model may be explained by two reasons: (1) the majority of human SCI lesions are incomplete [1], and [21] the perineal musculature of humans works via a similar mechanism, though distinctly different in timing [155].

Though distinct from mating in the presence of a receptive female, much knowledge has been gained about sexual circuitries through the *ex copula* sexual

reflexes, due to the ability to further explore the local spinal network and its supraspinal control [152] [150] [151] [122] [26] [59]. The first portion of the present dissertation work sought to expand upon previous studies using this reflex in a more clinically relevant SCI model and to improve its potential for use as a more sensitive tool for assessing therapeutic outcomes. Specifically, although the PDFR test has been well-defined in a complete transection SCI model [151], the effect of SCI contusion has, prior to this dissertation work, had limited exploration. Importantly, the PDFR has previously been visually scored by an observer, with penile engorgement and dorsiflexion given an intensity rating based upon rater observation [157] [26] [243] [244] [227]. While much can be learned by examining circuitries in all-or-nothing scenarios (i.e. transection), a greater understanding of the PDFR in incomplete lesions is necessary for translation and using subjective approaches such as the observation of penile engorgement and dorsiflexion intensity may be limiting the identification of subtle physiological changes when examining a therapeutic target.

As the lumbosacral penile reflex pacemaker is under inhibitory supraspinal influence from the MRF, in particular the nPGl [122] [26] [59], full transection leads to a very short latency to onset, as well as a much more intense response [151]. The reticulospinal projections involved with sexual function have been shown to reside bilaterally within the dorso-lateral quadrant of the spinal cord [222]. It was therefore hypothesized that latency and intensity of the PDFR is reliant upon the presence/absence of reticulospinal fibers traversing the contusion injury site (Chapter III). Utilizing kinematic analysis to examine the penile dorsiflexion, it was

determined that differences in hypersensitivity of the penile reflex between animals of varying injury severity indeed could be determined with reliable quantifiable measures. In Chapter III, an improved method of examining the PDFR is proposed, where the maximum angle of penile dorsiflexion, total penile even duration, and penile ascent speed can be objectively measured. These results support the idea of a discreet pathway of supraspinal control over the perineal musculature, where specifically, the ICM motoneurons were less sensitive in response to tonic stimulation of the DNP because of a greater supraspinal inhibitory influence. However, the differences in response of the PDFR between moderate and moderate-severe injury groups cannot be definitively stated as due to differing levels of descending inhibition within the scope of this study alone. Future studies utilizing kinematic analysis of the PDFR may be able to further support this hypothesis using anterograde tracing with biotinylated dextran amine (BDA) from the nPGl to the lumbosacral cord[58], where stereological analysis could determine likely differences between contusion injury severity animal groups in the descending inhibitory projection that are responsible for the control of the sexual reflexes [26].

The attributes of the PDFR are directly associated with the actions that occur during rat mating behavior, where penile dorsiflexion is synonymous with the penile 'flip' action that occurs prior to intromission (activation of the ICM), and glans cupping is representative of ejaculation (activation of the BSM) [187]. Eliciting the PDFR in a fully transected animal is examining an isolation of the reflexive sexual circuitry. However, in a mating behavior test, both intrinsic and extrinsic stimuli drive sexual behavior [22]. Therefore, it's necessary to consider the entirety of

physiological sexual function when examining the deficits in mating behavior after SCI. In Chapter IV, the differences in sexual function pre-vs-post injury are most logically and easily described as a discoordination between autonomic and somatic circuitries leading to the decreased ICP and poor mating behavior performance shown.

First, after SCI, ICP is significantly decreased during mounts, intromissions, and ejaculations, though the pressures are altered from baseline, suggesting vascular filling is occurring (the first stage of erectile response) [22]. High average pressures during mating events, as seen in pre-injury mating behavior testing and in sham laminectomy animals, supports ICM and BSM contraction aiding in retained ICP pressures (the second stage of erectile response) [22]. ICP plateaus are indicative of autonomic efferent input, whereas ICP peaks represent additional motoneuron input [12]. However, decreased pressures after SCI suggest the second stage of erectile response is insufficient in SCI animals in this study. Therefore, the first possible mechanism of action for decreased ICP and mating performance counts and durations is a discoordination of the somatic input onto the BSM and ICM. The BSM and ICM motoneuron pools have been shown to have 5-HT receptors [245] [246], Neuropeptide Y innervation [247], and noradrenergic terminals [248]. Additionally, oxytocinergic [249] [250], metenkephalin, substance P, noradrenaline, somatostatin, and serotonin immune-reactive fibers have been found in the dorsomedial and dorsolateral nuclei [251] [252] where the BSM and ICM motoneurons reside. Such neurotransmitters may be released from descending supraspinal inputs such as that of the nPGl [58] and raphe nuclei [65]. Additionally,

the pudendal motoneurons have dendrites reaching to the dorsal grey commissure and dorsal horn [219] [23] [253] [254] [255], which is the likely mechanism for autonomic and somatic coordination. Disruption of neurotransmitter outflow from supraspinal neurons after SCI likely alters the pudendal motoneurons innervating the perineal musculature. Indeed, Substance P and 5-HT immunoreactive staining was significantly depleted in the lumbosacral dorsomedial and dorsolateral nuclei after spinal transection [252].

The second mechanism through which the ICM and BSM may faulter in function after SCI is through decreases in hormonal influence. The perineal musculature is highly sensitive to reduction of androgens, where atrophy of the BSM and ICM can occur within 10 days of castration [256] [257] [258]. Additionally, castration causes a reduction in the soma size of the BSM and ICM motoneurons [259] [260]. Decreases in testosterone are seen in both humans [261] and animal models of SCI (Chapter II), and therefore, ICM and BSM after SCI may see a degree of atrophy and decreased soma size after SCI. Examination of the weights of BSM and ICM of SCI animals, along with retrograde tracing from the BSM and ICM and determination of motoneuron soma size in future studies could determine if decreases in testosterone after SCI impact perineal musculature function.

The third potential mechanism of decreased ICP and mating behavior after SCI is a dysregulation of autonomic coordination. Parasympathetic innervation arises from the sacral parasympathetic nucleus (SPN;Onuf's nucleus: L5-S1 in the rat), reaches the penis via the cavernous nerve, and is pro-erectile[167] [12]. This pro-erectile innervation is responsible for the NOS-mediated vascular filling stage of

erection[237, 238], as stimulation of the pelvic (and congruent) cavernous nerve causes penile engorgement [19] [17] [18] and increased ICP [262]. Sympathetic innervation of the penis originates in the intermediolateral cell column (IML;) and the dorsal gray commissure (DGC) (L1-L2 rat) and reach the penis via the paravertebral outflow (pudendal nerve); and the hypogastric nerve [13] [263] respectively [12]. With regards to erectile function, sympathetic innervation is overall considered to be anti-erectile, as hypogastric stimulation does not typically elicit erection, and stimulation of the paravertebral sympathetic chain causes detumescence [20]. Discoordination of the parasympathetic and sympathetic inputs in sexual dysfunction may be presenting as the decreased duration of mating behaviors and ICP in addition to perineal musculature dysfunction. Interestingly, spinal lesion of the sacral cord (and subsequent disconnect of the parasympathetic contribution to the penis) is thought to unmask “pro-erectile” fibers of the hypogastric nerve, where sexual simulation may cause a reduction in the tonic anti-erectile vasoconstriction of the penile vasculature [264] allowing for mild tumescence to occur. The inability to maintain an erection (seen in decreased duration and ICP in Chapter IV) after SCI may be caused by anti-erectile sympathetic fibers restricting vascular tone within the penis. Autonomic discoordination after SCI occurs in other pelvis organs whose innervation also originate in the SPN, such as detrusor-sphincter-dyssynergia in the bladder where disruption of the spino-bulbo-spinal pathway results in involuntary urethral closures during parasympathetically mediated detrusor contractions [265]. Alterations in the roles of autonomic regulation of erectile function could possibly be examined by ICP recordings during mating behavior testing and ex copula reflexes

after lesioning the differing sympathetic inputs (hypogastric nerve, and parvertebral outflow) and parasympathetic input (cavernous nerve) after SCI.

Additionally, neurons of the SPN, IML, and DGC all contain or are in close contact with fibers containing serotonin, dopamine, noradrenaline, adrenaline, and oxytocin. These neurotransmitters act through descending projections from supraspinal brain centers, respectively: the raphe nucleus, A11 cell group of the hypothalamus, locus coeruleus, nucleus subcoeruleus, and the paraventricular nucleus [12]. Brain levels of serotonin and dopamine are altered after SCI [266] [267], and cerebrospinal oxytocin has been shown to increase in the SCI dog [268]. Therefore, yet another mechanism for altered autonomic discoordination may be through the altered regulation of these neurotransmitters implicated in the central control of erection.

Vascular filling is the first step in proper erectile function, and therefore, it is important to consider changes in penile vascular function after SCI in regard to overall erectile function. Cardiovascular changes after SCI include heart rate, arterial blood pressure, and peripheral vascular changes [269] [270] [271] [272] [273]. Vasculogenic erectile dysfunction is the most common cause of organic erectile dysfunction [274]; however, the role of the penile vasculature in sexual dyfunction after SCI has yet to be fully examined in human or animal models. In Chapter IV, vascular filling is likely occurring after SCI, as seen in the plateau departures from baseline in the absence of peak pressures reached. To further determine the parasympathetic vascular role of ICP during mating behavior testing and the *ex copula* sexual reflexes in SCI animal models, the BSM and ICM can be removed,

therefore removing the ability to reach stage 2 of erectile function: rigidity. Through the course of this dissertation work, penile vascular ultrasound during the PDFR was attempted but unsuccessful. Future studies could examine penile vascular ultrasound during stimulation of the parasympathetic penile innervation (cavernous nerve) after SCI to further determine the effect of SCI on penile vasculature.

Ejaculation is comprised of two stages, emission and expulsion, and again, is a result of sympathetic, parasympathetic, and somatic input [46]. Coordinating these inputs is a spinal ejaculation generator that resides at the L3-4 cord in the rat [193] [194]. Here, lumbospinothalamic neurons integrate autonomic nuclei, pudendal motoneuron and genital afferent sensory input while receiving supraspinal input from the nPGl, MPOA, and PVN [194]. Though this dissertation work has mainly focused on erectile function, the capability of ejaculation after SCI was examined in Chapter IV, where 16% of animals with WMS less than 20% and 33% of animals with WMS greater than 20% were capable of ejaculation. Overall, decreased ejaculations may foremost be attributed to fewer intromissions and fewer intromissions of appropriate rigidity to allowing for a reach of ejaculation. Decreased genital sensory feedback to spinal ejaculatory centers may also be inhibiting the ability to ejaculate; however, intact rats who are unable to intromit due to perineal musculature removal are still capable of ejaculation, though the amount of genital stimulation from penis-to-female perineum is unknown [187]. Approximates 95% of SCI males with lesions caudal to T10 (approximately 25% of SCIs [1]) have disrupted ejaculation [36] [37] [38] [71]. Compromise of sympathetic outflow of pro-ejaculatory fibers responsible for seminal emission and bladder neck closure, along with sacral control of perineal musculature

responsible for expulsion can lead to anejaculation, retrograde ejaculation, or dribbling ejaculation. ICP increases during ejaculation prior to injury and in sham animals are due to pulsatile contractions of the BSM and ICM during expulsion phase [207]. Therefore, the decreases seen in ICP when ejaculation did occur after SCI are likely due to discoordination of the autonomic and sympathetic innervation between the ejaculatory center of the L3-L4 rat spinal cord [72] and the penis and perineal musculature as discussed above.

This dissertation work also examined the effects of ABT on sexual dysfunction after SCI, where SCI contusion rats showed an increase in sexual function as measured by the PDFR and BSM EMG to stimulation of the DNP. SCI males undergoing weight-supported stepping on a treadmill have shown a significant increase in sexual desire, though anecdotally reported changes in erectile function were not detectable by the IIEF questionnaire used [170]. Improvements in other autonomic functions have been reported from studies of the weight-bearing/limb placement/sensory input modulation of the lumbosacral cord [168] [169] [170] [190] [191] [162] [171]. Plasticity in the lumbosacral cord after SCI may be driven by propriospinal and other afferent input to impact the autonomic and motor output of the local circuitry responsible for sexual function [170] [212] [213]. Increased extensor activity has been shown to inhibit external urethral sphincter activity [275] [276] and step training has been shown to significantly increase soleus muscle EMG amplitude [169]. Perhaps extensor activity is similarly altering BSM and ICM activity, as they are also innervated and driven by pudendal motoneurons. An inhibition of the BSM and ICM hyperexcitability after SCI (as displayed in Chapter II and Chapter

III PDFR testing) may allow for the proper coordination of the musculature to correctly perform the role of enhancing and maintaining rigidity in erection and forceful expulsion of seminal fluid in ejaculation.

Additionally, as contusion injury is anatomically incomplete, there is potential for strengthening of intact residual fibers crossing the lesion epicenter with ABT. Treadmill running has been shown to attenuate synaptic stripping after sciatic nerve injury [277] [278]. Descending noradrenergic projections from the locus coeruleus modulate spinal motoneurons [279], and therefore, it is believed treadmill running activates the locus coeruleus which in turn provides noradrenergic-mediated maintenance of synaptic inputs of injured motoneurons [277]. As motor neurons of the SPN are in contact with fibers containing noradrenaline [12], it is feasible that ABT may increase sexual function via locus coeruleus activation through these residual fibers, and thus, increase noadrenaline mediated modulation of BSM and ICM motoneurons. Other neurotransmitters implicated in sexual function may also be enhancing the activity of spino-bulbo-spinal pathways and altering sexual function after ABT. For instance, exercise also alters brain serotonin levels [280] which may translate into alterations of pudendal motoneuron functioning. Neurotoxic lesions of the serotinergic system causes a similar increase in *ex copula* reflexes as seen in spinal lesions [26] [58] [281], and 5-HT agonists have been shown to have proerectile effects [282] [283] [284] [285]. However, pharmacologically induced increases in serotonin can cause ejaculatory dysfunction [286]. As the role of serotonin on sexual function is dependent on 5-HT receptor subtype involved [287] and multiple receptor subtypes are present throughout the sexual function circuitry

[12], alterations in serotonin levels post-injury and post-therapy may provide another mechanism of supraspinal influence of this circuitry.

Increases in sexual function were also seen in the metabolic and exercise control group that underwent forelimb only stepping training regimen in Chapter II. While animals having undergone task specific stepping and hindlimb weight bearing saw the greatest increases in sexual function with ABT after SCI, exercise effects in the SCI + FT animals cannot be ruled out. Propriospinal inter-enlargement pathways that mediate interlimb coordination for locomotor function in the rat could induce adaptive changes to neural networks within the lumbosacral cord [168] [169]. Additionally, aerobic exercise has been shown to improve erectile function in patients outside of SCI, where improved cardiac output allows for an increase in nitric oxide in the penile vasculature [288] [289]. Hormone modulation of exercise also cannot be overlooked [290], though in this study urine testosterone was unchanged in the rats. However, SCI men undergoing arm-crank exercise saw increases in testosterone, which could theoretically improve sexual function [206]. Future studies may examine the role of exercise effects versus neuronal plasticity due to somatosensory input in sexual function recovery after SCI by examining cardiac output as an additional exercise control or using the same sexual dysfunction outcome measures in another somatosensory plasticity model, such as tibialis anterior stimulation [211].

Though differences between ABT training groups was significant, the differences were modest. Future studies examining the role of ABT after SCI on sexual function, or any other potential therapeutic target of sexual dysfunction of

SCI, could benefit from utilizing the more quantifiable data provided by using PDFR kinematic analysis and/or ICP recording using a telemetric pressure catheter.

As previously stated, though this dissertation research does not focus on sexual dysfunction in females with SCI, such dysfunction remains an important quality of life issue amongst SCI women [6, 7]. The vaginal answer to male ICP as a measure of sexual function would be vaginal photoplethysmography [106]. Vaginal photoplethysmography can be used in both humans and animal models and is a well-established method for determining sexual arousal [74] [76] [86] [87] [106] [119] [120] [291]. Future studies examining the effects of SCI on sexual function should examine the relationship with injury severity as measured by WMS. Interestingly, due to the vagal role in the female genitalia-brain pathway [92], the severity of SCI may not play as significant of a role in sexual arousal ability in female animal models. Vasocongestion in response to sexual stimulation as measured by photoplethysmography would likely be decreased after SCI, however vagal influence may attenuate severe dysfunction as seen in male animal models. The effect of SCI on the female perineal musculature is likely similar to that of male perineal musculature, where the BSM and ICM are critically important in clitoral erection and blood retention. Such roles of female perineal musculature in regards to their role in sexual function in animal models has been much less studied compared to the male perineal musculature. Future studies examining ABT as a therapeutic target for sexual dysfunction after SCI would benefit from examining a potential therapeutic benefit to females, as well.

Overall, coordination of sexual function is an elaborate dance of autonomic and somatic function modulated and implemented at both the spinal and supraspinal levels. Residual fibers at the lesion epicenter left intact after a contusion injury are able to relay descending and ascending information and therefore influence the autonomic and somatic regulators of sexual function. The most studied pathway for supraspinal cooradination is the reticulospinal pathway [122] [26] [59]. This pathway projects through the dorsolateral quadrant of the spinal cord [222], and in the model of contusion injury used in this dissertation work, damage to this pathway varies with the severity of injury. In Chapters III and IV, measures of sexual function are correlated with the amount of WMS. As a less severe injury (example from this work: an injury with greater than 20% WMS) leaves a wider rim of white matter extending more medially, it is likely the reticulospinal pathway is partially spared and is capable of exerting greater influences on the spinal sexual reflex circuitries (shifting more closely toward intact control levels of function). It is important to note that this is a bilateral pathway, and for descending inhibition from the MRF to be lost, both ipsi- and contralateral reticulospinal pathways need be damaged [122]. Strengthening of these residual fibers through therapies such as ABT may allow for further improvements of coordination of the autonomic and somatic functions necessary for sexual function.

In conclusion, this dissertation research presents two improved methods of examining sexual dysfunction after spinal cord injury in a rat contusion SCI animal model: kinematic analysis of the PDFR, and real-time monitoring of ICP during mating behavior testing using a telemetric pressure transducer. Additionally, this

work demonstrates a significant improvement in sexual function as measured by *ex copula* sexual reflexes in a clinically relevant SCI animal model. Alterations in the descending and ascending pathways responsible for coordination of sexual function after SCI leads to discoordination of autonomic and somatic inputs onto the erectile and ejaculatory centers of the spinal cord and the perineal musculature responsible proper sexual function. Strengthening of the residual reticulospinal pathways and modulating plasticity of local circuitry via ABT will likely improve sexual function after SCI. While this work contributes to the field of sexual function after SCI, much is still unknown regarding the mechanisms by which the various aspects contributing to sexual dysfunction after SCI occurs. Future studies including those aimed at determining the effectiveness of therapeutic targets for this top priority quality of life issue may benefit from utilizing the quantitative measures examined in this work.

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CURRICULUM VITA

Casey Steadman
1840 Ekin Ave
New Albany, IN 47150
662.769.5556
casey.j.steadman@gmail.com

Education
Ph.D. – Anatomical Sciences and Neurobiology
University of Louisville, Louisville, KY
June 2019
GPA: 3.97/4.00

M.S. - Biomedical Engineering
Mississippi State University, Mississippi State, MS
August 2014
GPA: 3.63/4.00

B.S. - Biological Engineering
Mississippi State University, Mississippi State, MS
May 2012
GPA: 3.17/4.00

Research Experience
University of Louisville Louisville, KY
Graduate Researcher; Advisor: Charles Hubscher, PhD
Aug 2014 – Present

- Studying the impact of locomotor training on sexual function post-spinal cord injury

University of Mississippi Medical Center Jackson, MS
Graduate Researcher; Advisor: Lique M. Coolen, PhD
Jan 2013 – May 2014

- Studied the effect of prenatal exposure to excess testosterone on reproductive and metabolic function in a sheep model for Polycystic Ovarian Syndrome

Leadership

Mentor to Undergraduate Student March 2018 - Present
ASNB Graduate Student Council Representative January 2017- July 2018

SfN – Louisville Chapter Outreach Liaison May 2016 – July 2018

- Member of the Organizing Committee for annual Brain Day Science Celebration at the Kentucky Science Center
- Member of the Planning Committee for our local Neuroscience Day
- Organized our Chapter's participation in:
  - NanoDays at the Kentucky Science Center
  - Walk MS
  - Bike MS
  - March for Science Louisville
  - Eat! Drink! Do Science! at the Kentucky Science Center
  - Holiday Food Drive with Dare to Care Food Bank
- Hosted:
  - Lab tours for Northwest Kentucky Area Health Education Center students
  - Shadow Day for local high school students

KAS Monthly Column Contributor Nov 2016 – April 2017
Science Policy and Outreach Group President Aug 2016 – July 2017
Anatomy Seminar Class Representative Jan 2016 – April 2016
Graduate Teaching Assistant Academy student Aug 2015 – April 2016
Theta Tau Prof Engineering Fraternity Housing Corp April 2012 – April 2015
Theta Tau Professional Engineering Fraternity January 2009 – May 2012

Publications

Steadman CJ, Vangoor SS, Hubscher CH. Telemetric monitoring of penile pressure during mating in rats with different spinal cord contusion injury severities. In preparation. (2019)

Steadman CJ, Vangoor SS, Morehouse JR, Hubscher CH. Kinematic analysis of the penile dorsiflexion reflex. In preparation. (2019)

Steadman CJ, Hoey RF, Montgomery LM, Hubscher, CH. Locomotor training alters penile reflex responses in a rat model of spinal cord injury. In Review. (2019)

Gumbel, JH, Steadman, CJ, Hoey, RF, Armstrong, JE, Fell, JD, Yang, CB, Montgomery, LR, Hubscher, CH Activity-based Training on a Treadmill with Spinal Cord Injured Wistar Rats. J. Vis. Exp. (143), e58983, doi:10.3791/58983 (2019).

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Poster Presentations

Telemetric monitoring of penile pressure during mating in rats with different spinal cord contusion injury severities. Casey J. Steadman, Charles H. Hubscher. University of Louisville Neuroscience Day, 2019. 2nd Place.

Telemetric monitoring of penile pressure during mating in rats with different spinal cord contusion injury severities. Casey J. Steadman, Charles H. Hubscher. Society for Neuroscience meeting, San Diego, CA, 2018. Presentation number 568.01.

Locomotor training alters penile reflex responses in a rat model of spinal cord injury. Casey J. Steadman, Robert F. Hoey, Lynnette R. Montgomery, Charles H. Hubscher. University of Louisville Neuroscience Day, 2018.

Locomotor training alters penile reflex responses in a rat model of spinal cord injury. Casey J. Steadman, Robert F. Hoey, Lynnette R. Montgomery, Charles H. Hubscher. University of Louisville Graduate Student Research Conference, 2018.

Locomotor training alters penile reflex responses in a rat model of spinal cord injury. Casey J. Steadman, Robert F. Hoey, Lynnette R. Montgomery, Charles H. Hubscher. Society for Neuroscience meeting, Washington, D.C., 2017. Presentation number, 578.15.

Locomotor training alters penile reflex responses in a rat model of spinal cord injury. Casey J. Steadman, Robert F. Hoey, Lynnette R. Montgomery, Charles H. Hubscher. University of Louisville Neuroscience Day, 2017.

Insulin sensitizers block effects of prenatal testosterone excess in female sheep on expression of tyrosine hydroxylase, insulin and androgen receptors in the ventral tegmental area. C.J. Steadman, M.N. Lehman, V. Padmanabhan, L.M. Coolen. Society for Neuroscience meeting, Washington, D.C., 2014. Presentation number, 256.21.

Exposure to prenatal testosterone masculinizes dopamine expression in the ventral tegmental area in sheep: effects of postnatal treatments with flutamide or rosiglitazone. C.J. Steadman and L.M. Coolen. University of Mississippi Medical Center Neuroscience Research Day, 2014.

PRESENTATIONS

Casey J Steadman. “Locomotor training alters penile reflex responses in a rat model of spinal cord injury.” Neuroscience Day Datablitz. April 13, 2017.

Casey J Steadman. “Turned on: the neuroscience of sexual function.” Kentucky Science Center’s Scientific Proofs. March 8, 2016. Louisville, KY.

Membership

Kentucky Academy of Science	April 2016 – Present
Louisville Chapter Society for Neuroscience	August 2014 – Present
Society for Neuroscience	May 2014 – Present
Theta Tau Professional Engineering Fraternity	January 2009 – May 2012
Phi Mu Fraternity	August 2008 – May 2010
Alpha Lambda Delta Honor Society	Spring 2009

Scholarship and awards

Most Outstanding Trainee (Louisville Chapter of SfN)	April 13, 2018
Graduate Student Research Conference Poster Session 1st Place	March 3, 2018
Graduate Assistantship	August 2014 - Present
Graduate Assistantship	August 2012 – December 2012
Freshman Academic Excellence Scholarship	August 2008 – May 2012
Dean’s Scholar	August 2011 – May 2012
President’s Scholar	August 2008 – December 2008

Research Skills

Animal Behavior

- Skilled in performing mating behavior analysis in rat animal model
- Experienced in *ex copula* sexual reflexes, including kinematic analysis of reflex
- Experienced in locomotor step training

Telemetric Pressure Transducer Analysis

- Utilized LabChart to document mating behavior events
- Created Microsoft Excel Macro to analyze partial and full pressure erectile events

Animal Surgery

- Telemetric pressure transducer penile implantation
- Rat ovariectomy
- Rat estradiol implant
- Sheep progesterone intrauterine device placement
- Sheep estradiol implant

Electromyography

- Trained in terminal EMG recordings of bulbospongiosus muscle in response to stimulation of the dorsal nerve of the penis.
- Utilized Spike2 software for EMG analysis

Tissue Collection

- Experienced with phlebotomy, intravenous injections, laminectomies, tissue dissections, whole body perfusion, and tissue extraction in rat

- Experienced with phlebotomy, intravenous injections, head perfusion, and brain removal and fixation in sheep

Tissue Cutting

- Experienced with using a freezing microtome to section sheep and mouse tissue
- Experienced with using a cryostat to section rat tissue

Immunohistochemistry

- Experienced with immunohistochemistry techniques, including dual immunoperoxidase and triple immunofluorescence approaches

Microscopy and Imaging

- Skilled at bright-field and fluorescent microscopy
- Experienced with MicroBrightfield BioSciences Microscopy systems for analysis
- Adept at using a CCD camera to obtain high quality magnified tissue images
- Performed unbiased stereological analysis

Data Analysis

- Experienced with using statistical software to determine differences between experimental groups

Computational Skills

Proficient in:

- DSI Ponemah
- Labchart
- ImageJ
- MaxTRAQ
- MBF Bioscience Neurolucida
- MBF Bioscience Stereo Investigator
- MATLAB
- SigmaPlot
- Microsoft Office

Strong basis in:

- C Programming
- LabVIEW
- Mathcad