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Spinal cord injury induced cardiac decline and the limitations of exercise.

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SPINAL CORD INJURY INDUCED CARDIAC DECLINE AND THE LIMITATIONS OF EXERCISE

By

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B.S., Georgetown College, 2011
M.S., University of Louisville, 2015

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DEDICATION

This dissertation is dedicated to

My friends, my family and my mentors.

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I would first and foremost like to thank my parents who have always encouraged me in my endeavors towards success despite my ever-changing definition of the word. They have carried me during my darkest hours and stood beside me during the brightest. Most importantly, they have continued to love me despite the strain and stress that came along with completing my degree.

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ABSTRACT

SPINAL CORD INJURY INDUCED CARDIAC DECLINE AND THE LIMITATIONS OF EXERCISE

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Due to the improvement in acute care and overall management of spinal cord injury (SCI) the life expectancy and longevity of this population has increased substantially over the past decade. As such, a myriad of secondary injuries are being brought to light the majority of which relate to dysfunctions of the autonomic nervous system (ANS). Some of the most deleterious of which relate specifically to the decentralization of the cardiovascular (CV) system.

There is a general consensus that the level and the severity of SCI impacts the manifestations chronic cardiac abnormalities. For instance, cervical and high thoracic SCI are plagued by hypotension, bradycardia and reflexive autonomic imbalances. Furthermore, the extreme degree of immobility/inactivity experienced by the persons with SCI also has profound implications on cardiac dysfunction. Yet it has, thus far, been difficult to separate the relative contributions of the decentralized autonomic nervous system (ANS) and the immediate and profound inactivity to chronic cardiac abnormalities.

To date, the majority of pre-clinical studies investigating cardiac decline utilize full transection models, which do not anatomically represent the majority of clinical

SCIs. The vast majority of clinical SCI are incomplete injuries with some degree of residual function leading to wide range of locomotor, sensory and autonomic phenotypes. CV efforts have traditionally also been focused on understanding the most severe cardiac consequences such as autonomic dysreflexia (AD) while little attention has been given to changes in the heart itself. As such, the body of work presented in this dissertation sought to characterize a clinically relevant contusion model of SCI that results in persistent CV dysfunction and to investigate the effects of interventions on that model. Echocardiography, Dobutamine stress echocardiography and pressure volume assessments were utilized to gain insights into functional and structural remodeling and functional reserve following injury. Passive hind-limb cycling (PHLC) and swim (SWIM) training were also employed as an intervention to potentially reverse the observed cardiac decline. In order to further our understanding of the cascading effects of immobility/inactivity on SCI induced cardiac dysfunction, we then controlled spontaneous activity by limiting or increasing the amount of space available for animals to move. We found that the cardiac dysfunction elicited by a high-level, moderate contusion SCI was restored towards pre-injury levels with the administration of DOB; although the myocardial response to DOB was exaggerated chronically post-injury compared to pre-injury responses. Implementing PHLC and SWIM training revealed that neither exercise intervention had the ability to correct certain aspects of chronic cardiac dysfunction but PHLC attenuates systolic dysfunction. Finally, we found that hindering spontaneous activity further exacerbates SCI induced cardiac decline.

Our findings show that SCI disruption of the ANS results in diminished systolic function as demonstrated by the stark increase in flow metrics with DOB administration.

Furthermore, we show that when appropriate interventions are implemented acutely cardiac function can be favorably influenced. However, when spontaneous activity is hindered extreme inactivity compound the initial SCI and further exacerbates cardiac decline.

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CHAPTER I

INTRODUCTION

The Autonomic Nervous System and Cardiovascular Control

General organization of the autonomic nervous system

The autonomic nervous system (ANS) is comprised of two branches, sympathetic and parasympathetic, that work in tandem with one another to regulate homeostasis of visceral organs. The sympathetic nervous system (SNS) is primarily thought of as 'excitatory' and is thoracolumbar in its distribution while the parasympathetic system (PNS) is mainly 'suppressive' and has a craniosacral distribution. The majority of visceral organs are under control from both arms of the ANS. Exception to that rule is the vascular tree, where the majority of vascular beds only receive innervation from the SNS while the cavernous tissue of the male and female genitalia receive only parasympathetic innervation. Although the SNS and PNS reach their target organs via different pathways and have opposing actions there are several similarities between the two branches. Both the SNS and the PNS are considered a two-neuron system. The first population, preganglionic neurons, of the SNS and PNS are housed in the gray matter of the brainstem or spinal cord (SC) and must synapse onto the second population of neurons, postganglionic neurons, located in peripheral ganglia before synapsing onto their target organs. Both the SNS and PNS preganglionic neurons release the same neurotransmitter, acetylcholine (ACh), onto their postganglionic counterparts. By anatomic design, the

route preganglionic neurons travel to reach their target organs differs between the SNS and PNS. As such, the axons of SNS preganglionic neurons tend to be shorter than their postganglionic counter parts while preganglionic PNS neuron axons have a much longer path to travel than those of postganglionic neurons (Fig.1).

Sympathetic preganglionic neurons are housed in the SC within the intermediolateral cell column (IML), which only spans from the first thoracic spinal segment to first/second lumbar levels (T1-L1/2). Preganglionic axons exit the SC in the ventral root, which meets with the dorsal root to become a true spinal nerve. Preganglionic fibers will then exit the true spinal nerve via a white ramus to enter the paravertebral chain ganglia located lateral to the spinal column and within thoracic and abdominal cavities. Here preganglionic axons will do one of three things: 1) immediately synapse on postganglionic cell bodies housed in the sympathetic chain ganglia and then re-enter the spinal nerve via a gray ramus, 2) ascend or descend several segments within the sympathetic chain before synapsing on a postganglionic neuron or 3) exit as a preganglionic splanchnic nerve to reach paravertebral ganglia and visceral organs in other body cavities (Fig.2). Postganglionic fibers re-entering the true spinal nerve will be distributed to the body wall via dorsal or ventral rami to supply blood vessels and sweat glands. Sympathetic postganglionic neurons are mainly adrenergic releasing norepinephrine (NE) on their target organs unlike the PNS (Fig.1). The SNS is divided segmentally where different SC segments/levels house preganglionic sympathetic neurons destined to specific target organs and this distribution is topographically organized body cavity. For instance, the majority of sympathetic control to the heart is derived from the T1-T4 levels (Strack, Sawyer et al. 1988) while the vascular beds in the

abdomen are serviced by T6-T12 levels (Fig.3). The segmental organization of the ANS (SNS and PNS) is incredibly important when considering specific pathologies associated with differing levels of spinal cord injury.

The PNS has traditionally been referred to as being craniosacral in its distribution in the central nervous system (CNS) . Preganglionic cell bodies are localized in four nuclei of the brainstem (CN III, VII, IX, and X) and within the sacral segments of the spinal cord (S2-S4). The majority of the parasympathetic cranial nerves (CN III, VII, IX) only provide innervation to structures in the head and neck. These CN will synapse onto one of the four parasympathetic ganglia
(cilliary {III}, otic{IX}, pterygopalatine {VII} and submandibular {VII}) containing postganglionic cell bodies, which will then supply glands and viscera within the head and neck. The exception to that rule is CN X, which is not confined to the head and neck and provides parasympathetic innervation to 2/3 of the body. Cranial nerve X preganglionic neurons will travel through the body cavities and synapse onto intramural ganglia located within the walls of their target organs. Unlike the SNS, both preganglionic and postganglionic parasympathetic fibers are strictly cholinergic releasing ACh. It is important to note that the traditional ‘craniosacral’ organization of the PNS is now under scrutiny. It has been proposed by Espinosa-Medina and colleagues(Espinosa-Medina, Saha et al. 2016) that the autonomic neurons in the sacral SC are actually sympathetic due to how these preganglionic neurons are derived developmentally.

Autonomic control of the cardiovascular system

Like other visceral organs, the cardiovascular (CV) system is under dual (i.e. sympathetic and parasympathetic) innervation from the ANS. Sympathetic supra-spinal control to the cardiovascular end organs (heart and blood vessels) originates in the rostral ventral lateral medulla (RVLM) of the brainstem and descends within the SC.(Dampney 1994) The location of these descending axons is relatively conserved across species, from rodents to humans, and they descend within the dorsolateral funiculus (Reis, Morrison et al. 1988, Ruggiero, Cravo et al. 1989, Furlan, Fehlings et al. 2003) (Fig.4). Axons from the RVLM synapse onto sympathetic preganglionic neurons in the IML to influence both cardiac and vascular function. Preganglionic sympathetic neurons destined for the heart are housed in the T1-T5 (Strack, Sawyer et al. 1988, Krassioukov 2009) spinal segments, which will in turn exit the SC to reach their postganglionic counterparts. The majority of sympathetic postganglionic neurons with terminals onto the myocardium and sinoatrial (SA) node are found in the stellate ganglia—the joint inferior cervical and T1 ganglia. Sympathetic innervation to the vasculature is segmental such that preganglionic neurons supplying the upper body vasculature originate in the T1-T6 spinal segments while the lower body vasculature and the gut (which can be further divided) receive innervation from the T6-T12/L1-2 spinal segments. Parasympathetic fibers targeting the heart originate from the dorsal motor vagal nucleus (Machhada, Marina et al. 2016) and travel extra spinally. As the preganglionic Vagus nerve (CNX) fibers travel through the thorax they will join postganglionic sympathetic fibers of cardiopulmonary splanchnic nerves to form the cardiopulmonary plexus. Once cardiopulmonary splanchnic nerves meet the heart, preganglionic parasympathetic fibers synapse on intramural ganglia and become

postganglionic in the wall of the organ. Unlike the heart, the vasculature (except for urogenital vascular beds discussed above) receives only sympathetic innervation.

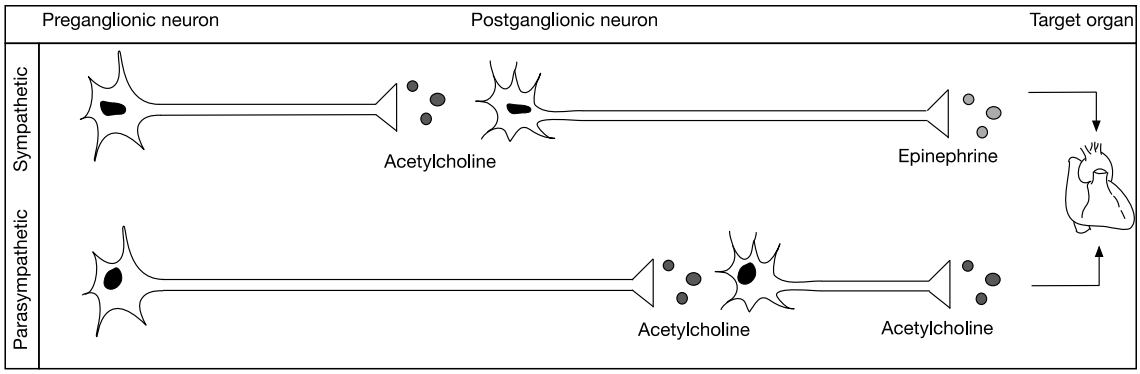


Figure 1. Organization of the autonomic nervous system pre and postganglionic neurons.

Comparison between preganglionic and postganglionic neurons of the autonomic nervous system. The ANS is comprised of the sympathetic and parasympathetic branches. Due to differences in the pathways these neurons travel to reach their target organs, the sympathetic preganglionic neurons are shorter than the parasympathetic while the sympathetic postganglionic neurons are longer than parasympathetic counter parts. All preganglionic neurons release Acetylcholine (ACh) as their neurotransmitter. Sympathetic postganglionic neurons release epinephrine.

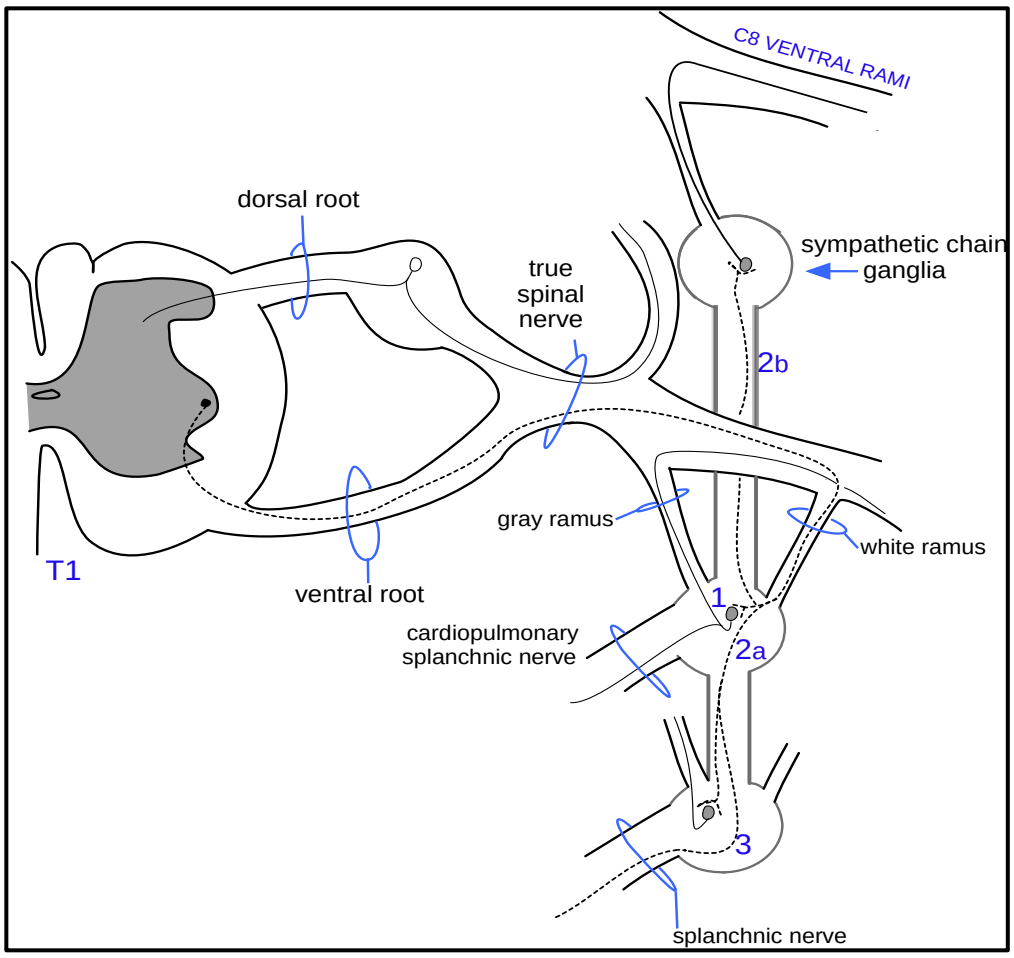


Figure 2. The pathways taken by sympathetic preganglionic neurons to become postganglionic.

Preganglionic neurons will do one of three things before synapsing on a postganglionic cell body. Preganglionic neurons will enter the sympathetic chain ganglia via a white ramus and can (1) immediately synapse on postganglionic cell bodies housed in the sympathetic chain ganglia at the same spinal or travel (descend (2a), ascend (2b)) several segments within the sympathetic chain before synapsing. Postganglionic neurons will then re-enter the spinal nerve via a gray ramus or exit the sympathetic chain as a splanchnic nerve destined for thoracic viscera. Preganglionic neurons can also (3) enter the sympathetic chain and exit without synapsing as a preganglionic splanchnic nerve targeting paravertebral ganglia and visceral organs in the abdomen.

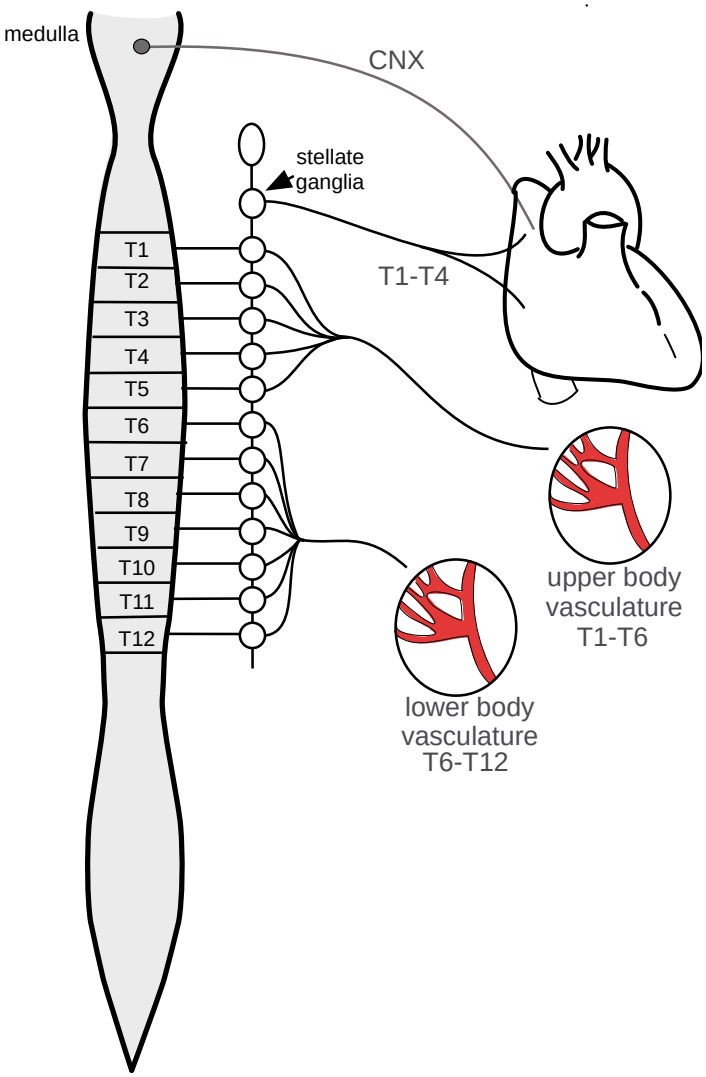
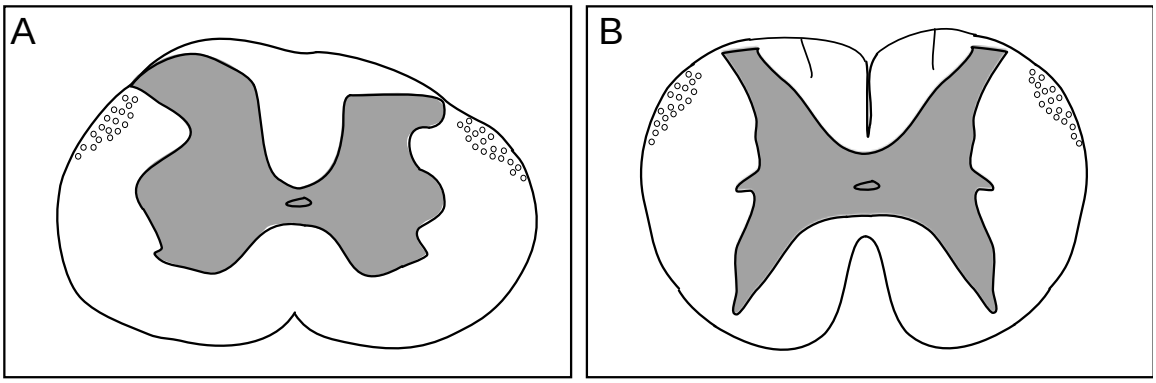


Figure 3. Segmental organization of the autonomic nervous system.

Sympathetic preganglionic neurons supplying the heart are housed in the thoracic level one-four (T1-T4) spinal segments, the upper body vasculature T1-T6 and the lower body vasculature T6-L1/L2. Figure adapted from Thieme Atlas of Anatomy Figure.50.1 (Gilroy and MacPherson 2016)



<u>Figure 4.</u> The descending vasomotor control form the rostral ventrolateral medulla.

Descending tracts travels within the dorsolateral funiculus of the spinal column traversing through the most lateral aspects of the thoracic one (T1) spinal segment (panel A) and lower thoracic levels (panel B) to reach preganglionic neurons in the intermediolateral cell column.

Cardiovascular Control following Spinal Cord Injury

Due to advances in the acute medical treatment of SCI and the overall understanding of injury consequences, the life expectancy of individuals with SCI has dramatically increased (reference). However, with increased longevity, secondary injuries that were not previously recognized are becoming prevalent. Some of the most severe and debilitating secondary complications associated with SCI are the alterations in autonomic CV control. Furthermore, there is a lesion-dependent impairment in CV function and severe deconditioning combined with the initial locomotor and sensory losses predisposes individuals with SCI to early onset cardiovascular disease (CVD) and abnormal CV control compared to their able-bodied counter parts (Krassioukov and Claydon 2006). Understanding how these deficits manifest over time is incredibly important when considering the implementation of interventions to prevent or reverse cardiac abnormalities.

Acute and sub-acute time period

Immediately following injury there is a period of spinal shock characterized by marked reductions in sensory, locomotor and reflexive functions below the level of the injury (Ditunno, Little et al. 2004). In addition, instances of bradycardia and severe hypotension (i.e. neurogenic shock) are persistent for the first few days post-SCI and are most prevalent in individuals sustaining cervical injuries (Guly, Bouamra et al. 2008, Mallek, Inaba et al. 2012, Summers, Baker et al. 2013). Neurogenic shock can last up to 5 weeks post-SCI (Furlan, Fehlings et al. 2003, Krassioukov and Claydon 2006) but has also been reported to resolve within the first four days following injury (Lehmann, Lane

et al. 1987). Reductions in BP acutely following injury are such that vasopressive therapy is often necessary (Furlan, Fehlings et al. 2003). Current guidelines suggest a maintenance of blood pressure at a minimum of 85mmHg and clinical studies have shown vasopressive therapy may be required for up to five weeks (Furlan and Fehlings 2008). Bradycardia has been reported in 17-77 percent of individuals with cervical SCI, and only 0-13 percent of thoracolumbar injuries (Hector, Biering-Sorensen et al. 2013). Implementing therapies during the acute and sub-acute time period has widely been avoided due to a high occurrence of multiple injures sustained along with the SCI as well as the prevalence of orthostatic hypotension and altered basal hemodynamics (Illman, Stiller et al. 2000). However, from pre-clinical studies West *et al*. 2014 has shown CV benefits of beginning intervention early post-injury.

Chronic spinal cord injury

In addition to the maladaptive nervous system reorganization and neuronal plasticity that occurs acutely post-SCI (Krenz and Weaver 1998, Krenz and Weaver 1998), the decentralization of the ANS eventually leads to overall CV system wide breakdown and dysregulation, which manifests chronically. From pre-clinical and clinical studies, altered hemodynamics (Wecht, de Meersman et al. 2000, Wecht, Zhu et al. 2013, Wecht, Weir et al. 2015, Squair, West et al. 2016), cardiac atrophy (Kessler, Pina et al. 1986, Nash, Bilsker et al. 1991), systolic dysfunction(Kessler, Pina et al. 1986, West, Bellantoni et al. 2013, West, Gee et al. 2015, West, Crawford et al. 2016) and increased risk of reperfusion arrhythmias (Lujan, Palani et al. 2010, Lujan, Janbaih et al. 2012) have all be widely documented in chronic SCI. These CV abnormalities are a result of

both the decentralization of the ANS from descending sympatho-excitatory control originating in the RVLM (Krassioukov and Claydon 2006, Krassioukov 2009, Squair, West et al. 2016) as well as significant cardiac unloading secondary to sympathetic decentralization of the peripheral vasculature (West, Mills et al. 2012, West, Alyahya et al. 2013), reduced circulating blood volume (Houtman, Oeseburg et al. 2000), and a loss of muscle and respiratory pumps (Faghri and Yount 2002). Ultimately, the initial insult compounded with extreme inactivity manifests chronically with cardiac abnormalities that differ according to lesion level and severity.

Autonomic dysreflexia

Arguably one of the most life threatening CV consequences of SCI is an inappropriate vascular response to stimulus, either noxious or non-noxious, below the level of the lesion, known as autonomic dysreflexia (AD). Autonomic dysreflexia is a hypertensive crisis where intense vasoconstriction is elicited by an unregulated sympathetic response to sensory stimulation (Krassioukov 2009) and is classically defined as an increase in mean arterial pressure (MAP) greater than 20-30mmHg above basal levels (Krassioukov, Warburton et al. 2009). Deleterious pressure increases can occur in excess of 40 times per day (Hubli, Gee et al. 2015, West, Popok et al. 2015). If the symptoms of AD are not recognized and are left untreated episodes can lead to myocardial infarct, stroke and even death (Pine, Miller et al. 1991, Eltorai, Kim et al. 1992, Valles, Benito et al. 2005, Dolinak and Balraj 2007). From pre-clinical studies it is suggested that the sprouting of central branches of calcitonin gene related peptide (CGRP) immunoreactive fibers into lamina III and IV of the lumbar dorsal horns

contributes to the severity and number of AD episodes (Krenz and Weaver 1998, Krenz, Meakin et al. 1999, Laird, Carrive et al. 2009). As such, the constant swinging of blood pressure experienced by individuals with SCI is thought to contribute to the increased risk of heart disease in addition to the consequences at acute onset.

Orthostatic hypotension

Persons with SCI also suffer from the inability to vasoconstrict, or maintain blood pressure, in peripheral vasculature during postural changes resulting in orthostatic hypotension (OH) (Mathias 1995, Claydon and Krassioukov 2006). The occurrence of OH is classified as a 20 mmHg drop in systolic blood pressure or a 10mmHg drop in diastolic pressure. When OH occurs, blood momentarily pools in the peripheral vasculature and cannot be rapidly redistributed ultimately leading to decreased venous return and cerebral hypoperfusion (Gonzalez, Chang et al. 1991). Thus individuals experience light headiness, fatigue, blurred vision and difficulty with breathing (dyspnea) (Frisbie and Steele 1997, Mathias, Mallipeddi et al. 1999, Sclater and Alagiakrishnan 2004). The loss of sympathetic vasomotor control (Claydon, Hol et al. 2006), low resting catecholamine levels (Schmid, Huonker et al. 1998) and lack of increased catecholamine levels during head-up tilt (Mathias, Christensen et al. 1980) are all proposed mechanisms contributing to the pathophysiology of OH.

The effect of lesion level in chronic spinal cord injury

The relationship between lesion level and the extent of cardiovascular dysfunction is established in both pre-clinical and clinical studies (West, Mills et al. 2012, Wecht,

Zhu et al. 2013, Zhu, Galea et al. 2013, Wecht, Weir et al. 2015). Individuals with high level lesions, cervical and high thoracic (T6 and above) experience the most severe cardiac abnormalities. The presence and severity of CV abnormalities is a direct consequence of the disruption of descending control from the RVLM (discussed above). High-level injuries decentralize the majority of supraspinal control to SNS preganglionic neurons targeting the heart and vasculature, whereas low-level injuries spare that descending control and sympathetic preganglionic neurons targeting the heart and upper body vasculature remain intact. Although there are most likely significant changes in the PNS, this area of research is relatively unstudied.

Traditionally the classifications of quadriplegic, tetraplegia or paraplegic derived from the American Spinal Injury Association (ASIA) scale are used to group data from individuals for lesion level comparisons. Using these divisions is tempting since there are often low numbers of subject participants in clinical studies. However, since ASIA classifications are based on neurologic completeness of the injury they are not necessarily representative of the ANS disruption (Strack, Sawyer et al. 1988, Teasell, Arnold et al. 2000). Recently, classifications that more accurately represent the segmental organization of the ANS such as cervical (C; C1-C8), high thoracic (HT; T1-T6) and low thoracic (LT; T7-L5) are being used to group data when associating CV function with lesion level (West, Mills et al. 2012).

Cervical patients have been characterized with low resting HRs (Dixit 1995, Krassioukov and Harkema 2006, Wecht, Zhu et al. 2013, Zhu, Galea et al. 2013, Wecht, Weir et al. 2015), reduced basal blood pressure (Krassioukov and Harkema 2006, West, Campbell et al. 2012, Wecht, Zhu et al. 2013, Zhu, Galea et al. 2013, Wecht, Weir et al.

2015) and attenuated responses to instances of increased cardiopulmonary demand (i.e. peak responses) (Schmid, Huonker et al. 1998, Currie, West et al. 2014, West, Gee et al. 2015). This group also has the lowest levels of circulating catecholamine, a consequence of disrupted sympathetic control to the adrenal medulla (Schmid, Huonker et al. 1998). In addition, persons with cervical SCI are incredibly prone to episodes of AD that may even occur during the acute/sub-acute time period following injury (Silver 2000, Krassioukov, Furlan et al. 2003). Episodes of AD are more deleterious to cervical patients due to the profound, persistent hypotension. Raising BP to even normal (able bodied) levels can be deleterious.

Due to the heterogeneity of HT injuries, a clear characterization of CV deficits is not well defined. For example, an injury at the T1-T2 level would most likely present with CV deficits similar to the C population because of the complete disruption of supraspinal control of the SNS outflow targeting the heart and vasculature. However, injuries at the T4-T6 level could spare, depending on the severity and spread of the injury, innervation to the heart and upper body vasculature. Therefore, when considering all HT injuries together, this group typically does not appear statistically different from their LT counterparts. Schmid *et al.* 1998 reported that HT (defined as T1-T4) had lower resting HR compared to mid and LT (lesions below T5) groups. Contradictory to those findings, Wecht *et al.* 2013 reported that HT persons evaluated within their study had increased instances of tachycardia, which was similar to previous data published from their lab (Rosado-Rivera, Radulovic et al. 2011). It has been proposed that an increased basal HR may be compensating for the reduced stroke volumes (SV). The discrepancies in these findings may also be attributed to non-uniform group organization where Schmid

defines HT as T1-T4 while Wecht defines HT as T1-T6. Although it seems like an inconsequential difference, the majority of preganglionic neurons targeting the heart are housed at the T2 spinal segment. Thus, the direct disruption of sympathetic cardiac control may be what is causing the differences observed in the groupings. High thoracic groups are reported to have lower systolic and diastolic BP compared to LT group counterparts. Cardiovascular parameters in LT population appear to be similar to those of able-bodied counterparts most likely due to a sparing of the majority of SNS supraspinal control to the peripheral vascular tree. Reports of HR and BP show that injuries at the LT level appear to have near normal control over cardiac end organs (heart and vasculature). However, when placed under instances of high cardiopulmonary demand, such as field tests and arm crank exercise LT have attenuated peak responses (Hopman, Dueck et al. 1998, Currie, West et al. 2016).

Neurologic and Autonomic Completeness

The majority of SCIs are deemed incomplete where some degree of residual locomotor and sensory function remains. Yet, the relationship between injury severity (completeness) and CV function is not as well established as the relationship between lesion level and CV decline. This is in part due to the traditional definition and evaluation of neurological completeness of the injury, which evaluates the loss of motor and sensory function. Neurological completeness is assessed with a neurological exam that is in accordance with the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) (Kirshblum, Biering-Sorensen et al. 2014). These standards outline the evaluation of function remaining in dermatomes and myotomes, thus targeting

corticospinal and spinothalamic (locomotor and sensory) tracts. Visceral organ function is not evaluated in the current guidelines and thus the extent ANS disruption is neglected. Recently, several research groups have become more interested in autonomic completeness and the decentralization of the ANS due to the prevalence of secondary injuries that has been emerging with increased life expectancy. In 2012 an additional standard for assessing autonomic function, the International Standards to Document remaining Autonomic Function (ISAFSCI) (Contributors, Krassioukov et al. 2012) was developed as a push towards raising awareness of autonomic abnormalities. However, these standards do not provide an assessment of autonomic function but rather simply documentation of autonomic deficits. Furthermore, since its inception, less than 50% of people claim to be confident in administering the ISAFSCI even after completing a workshop explaining the use and implementation of the ISAFSCI (Round, Park et al. 2016). Currently, the most commonly used method to evaluate autonomic function is the sympathetic skin response (SSR), which records changes in skin conductance after activation of sweat glands along surfaces with a high density of eccrine glands. For example, the median nerve is stimulated along the palmar surface of the hand and peroneal nerve is stimulated along the plantar surface of the foot to target all levels of the IML. The integrity of preganglionic and postganglionic neurons is essential for a SSR (Yokota, Matsunaga et al. 1991, Cariga, Catley et al. 2002). The extent of cardiovascular impairment correlates with the SSR recordings. For instance, the presence or absence of OH and blood pressure abnormalities can be predicted from palmar SSR (Claydon and Krassioukov 2006).

Acute and sub-acute SCI cardiovascular changes

To date, only a few clinical studies have assessed the effects of injury completeness on CV function during the acute/sub-acute period and there is no general consensus on the presentation of CV abnormalities such as altered HR and BP. The presence of neurogenic shock acutely post-SCI most likely confounds CV measurements. Due to lack of statistical analyses and small samples sizes, there is no agreement whether individuals with complete SCI show enhanced CV dysfunction compared to incomplete counterparts.

Chronic SCI

When considering only the neurological completeness of the injury rather than stratifying based autonomic completeness, it is difficult to find meaningful CV differences. For instance, based on neurological completeness of the injury cervical and high thoracic SCI display similar resting BP between incomplete and complete injuries (Grimm, De Meersman et al. 1997, Rosado-Rivera, Radulovic et al. 2011, Sahota, Ravensbergen et al. 2012). However, clinical studies also suggest that neurologically incomplete cervical SCI experience fewer episodes of AD. Curt *et al.* 1997 reported that 91 percent of neurologically complete cervical individuals experiences AD episodes with urodynamic assessment while only 27 percent of cervical incomplete injuries devolved AD in response to the stimulus (Curt, Nitsche et al. 1997). In congruence with Curt and colleagues, Helkowski *et al.* (Helkowski, Ditunno et al. 2003) conducted a retrospective chart review and also found a reduced prevalence of AD in persons with neurologically incomplete cervical SCI compared to complete cervical SCI. In another study conducted

by Curt et al. 1996 (Curt, Weinhardt et al. 1996) half of the individuals with neurologically incomplete SCI exhibited no preserved palmar or plantar SSRs and all patients with AD lacked SSRs suggesting a discrepancy in the neurologic and autonomic completeness of injury. Furthermore, Claydon et al. 2006 (Claydon and Krassioukov 2006) found that 40 percent of individuals with a neurologically complete injury (ASIA A) exhibited some degree of SSR and that 30 percent of individuals with an AISA B, C, or D exhibited no preservation of SSR

Stratifying injuries based on autonomic completeness of the injury reveals differences in CV outcome measures. The SSR is primarily used to classify individuals for autonomic completeness of injury but other methods including measuring circulating levels of catecholamine and BP variability have also been used as markers of autonomic completeness (Sahota, Ravensbergen et al. 2012). Sahota *et al.* 2012 reported that incomplete SCI have higher SBP compared to complete injuries. Furthermore during instances of increased cardiovascular demand, such as head-up-tilt, incomplete SCI exhibit a larger increase in HR (Sahota, Ravensbergen et al. 2012). In addition to this, peak HR obtained during exercise is significantly greater in tetraplegia individuals with residual SSR responses (Currie, West et al. 2014).

Despite the prevalence of incomplete injuries, the majority of pre-clinical studies that investigate CV function typically utilize full transection models (Collins and Dicarlo 2002, Lujan and DiCarlo 2007, Laird, Carrive et al. 2009, Lujan, Palani et al. 2010, Lujan, Janbaih et al. 2014, West, Crawford et al. 2014). The use of these models is widespread because of the ease of conducting the SCI and the uniform nature of the injury. However, as mentioned previously, the majority of clinical injuries are

incomplete, leading to a wide range of locomotor, sensory and CV phenotypes. Therefore, full transection models are not anatomically representative of the clinical SCI population. To-date, only two pre-clinical studies have investigated CV outcomes in response to contusive SCI. Squair *et al.* 2015 administered several injury severities at the T3 spinal segment to evaluate the presence of a graded decrease in CV function with increasing injury severity. From this study it was demonstrated that the moderate (200kdyn) and severe (400kdyn) SCIs resulted in hypotension and increases in HR. Moderate and severe injuries also had stronger AD responses to colon distention, which was attributed to an increase in CGRP immunoreactivity in the SC dorsal horn. Nout and colleagues evaluated CV recovery between two injury severities under the influence of either pentobarbital or isoflurane anesthetics. (Nout, Beattie et al. 2012) In all SCI groups there was a persistent increase in HR from baseline, which is in agreement with clinical evaluations of CV function in HT lesions. (Wecht, Zhu et al. 2013) There was also persistent hypotension in the most severely injured animals, suggesting that the severity of the injury and disruption of preganglionic neurons relates to the observed CV function. Interestingly, neither body of work evaluated cardiac structure and functional deficits but focused on hemodynamics and HR changes. This is typical for the literature surrounding CV outcomes, where only the peripheral component of the CV system is evaluated.

The Influence of Exercise on Cardiac Function Following Spinal Cord Injury

It is likely that the extreme low levels of physical activity and fitness (due to wheel chair dependence) experienced by persons with SCI contributes to the three fold greater risk of developing early onset CVD compared to their able bodied peers. (Cragg,

Noonan et al. 2013) Other inactivity related diseases such as osteoporosis and type II diabetes are also prevalent in the SCI population(Garshick, Kelley et al. 2005) most likely due to increased incidences of obesity (Gupta, White et al. 2006, Gater 2007) and deposits of visceral adipose tissue (Edwards, Rankin et al. 1981), which is well known to be an independent risk factor for CVD (Despres, Moorjani et al. 1990). In addition significant dyslipidemia, characterized by depressed levels of high-density lipoprotein (HDL) (Bauman, Spungen et al. 1992, Bauman and Spungen 2008, Cowan and Nash 2010) and increased levels of low density lipoprotein (LDL) (Schmid, Halle et al. 2000). Interestingly the risk of developing CVD is heavily dependent on the level of the lesion, similar to the pattern observed with overall CV function. This is most likely due to the *profound* inactivity experienced following high-level injuries, which would ultimately culminate into the increased risk factors.

Exercise is a viable means to combat early onset CVD and improve cardiac decline. Despite the evidence that there is extensive cardiac remodeling, and a potential for it to be corrected with exercise, few studies have investigated this posit. Therefore, there is no general consensus on an ideal exercise or intervention that would positively influence CV recovery. Furthermore, it may very well be the case that therapies that are beneficial to other components of the CNS like the locomotor and sensory system may not affect the ANS in a similar manner.

Arm ergometry

Due to lower limb paralysis, exercise interventions have typically been limited to the upper body. However, there have only been a few reports of positive cardiac effects

associated with upper body exercise. Washburn *et al*. (Washburn, Savage et al. 1986) demonstrated that physically active men with SCI have better CV function then their physically inactive counterparts suggesting that chronic amounts of upper arm activity positively influences CV health. In addition, Huonker *et al.* (Huonker, Schmid et al. 1998) reported that long term training in wheelchair racing, basketball, and cross country sledding resulted in EDVs similar to non-trained able bodied individuals. It is important to note that the individuals from this study were all members of the German Paralympic teams may be predisposed to have higher resting function due an autonomic incomplete injury (Currie, West et al. 2014) and only eight of the individuals had injures between the T1-T5 spinal levels. Furthermore, there was no autonomic evaluation (SSR) performed during this study. Contrary to the findings put forth by Washburn and Huonker, several other studies have shown that upper-limb exercise is not sufficient to prevent the SCI induced cardiac decline.(Davis, Shephard et al. 1987, Nash and Jacobs 1998, Gates, Campbell et al. 2002, West, Campbell et al. 2012) A recent case study complete by West *et al.* 2015 demonstrated that when arm ergometry is coupled with passive lower limb exercise, improvements in cardiac flow indices such as stroke volume can be generated (West, Currie et al. 2015).

Functional electrical stimulation

Functional electrical stimulation (FES) cycling is one way to circumvent the limitations of upper body exercise. During FES, electrodes are placed over deinnervated muscles (i.e. quadriceps, gluteal and hamstring muscles) and movements of the joints are elicited via sequential electrical stimulation. Movement of the leg is used then to

manipulate an exercise device such as a bike. Reversal of obesity (Griffin, Decker et al. 2009) and lower limb muscle atrophy (Devillard, Rimaud et al. 2007) can be accomplished with this FES cycling. In the only study to date to investigate the cardiac effects of FES cycling, chronic SCI participants had an increase in heart mass by 35% and in the left ventricle internal diameter was normalized. These changes were attributed to both a pressure and volume loading of the heart from cycling (Nash, Bilsker et al. 1991). Although there appear to be cardiac benefits associated with FES, episodes of AD have also been elicited from this intervention (Ashley, Laskin et al. 1993). In addition to the adverse side effects sometimes associated with FES, the overall equipment is difficult to set up, not widely available to the SCI community and cannot be used by individuals with a history of spasticity (Cybulski, Penn et al. 1984).

Passive hind-limb cycling

There is growing interest in passive lower body exercise since a myriad of beneficial effects have been indicated from passive hind-limb cycling (PHLC) intervention. From pre-clinical studies, PHLC has been shown to maintain lower limb muscle mass (Houle, Morris et al. 1999), improve sensory function,(Hutchinson, Gomez-Pinilla et al. 2004) and decrease circulating inflammatory markers.(Sandrow-Feinberg, Izzi et al. 2009) Most importantly, it was recently reported that PHLC prevents cardiac decline, stops collagen deposition, improves metabolic markers and diminishes AD responses to CRD.(West, Crawford et al. 2014, West, Crawford et al. 2016) In addition, with isolated heart preparations, it was shown that PHLC groups pressure generated starling curves that were similar to control animals suggesting that PHLC stretches the

myocardium in such a way to maintain proper contractile function. It is important to note that the studies investigating PHLC and cardiac function were all completed with full transection models. Furthermore, unlike FES, passive hind-limb cycling does not require extensive set up and can be completed with much more simple equipment. During PHLC the limbs are mechanically rotated by either custom-made lower limb ergometers that do not extend joints beyond their optimum angle of extension or by physiotherapists that rotate the legs manually (Svensson, Siosteen et al. 1995).

Body weight supported treadmill training

Body weight supported treadmill training (BWSTT) is being used more and more often to aid CNS recovery and there has been success with the intervention in regards to locomotor recovery. Although these therapies improve locomotor recovery the effect of BWSTT on other systems is still poorly understood despite the evident possibility that manual manipulation of the lower limbs may be inducing CV effects. There are only a few studies that explore the effects of treadmill training on CV outcomes and they focus on the most deleterious CV measures such as AD and orthostatic intolerance rather than positive cardiac remodeling. Krassikouv and Harkema reported adverse effects of the training equipment itself where systolic and diastolic pressure are increased in response to the tight straps of the harness (i.e. noxious stimulus) (Krassioukov and Harkema 2006). In addition to this Ditor et al. (Ditor, Macdonald et al. 2005) reported that treadmill training may elicit orthostatic intolerance hallmarked by a decrease in MAP and a compensatory increase in HR elicited by the specific body positioning of the training. However, Ditor also reported that BWSTT improved function in lower-limb vasculature,

which would ultimately result in improved overall CV function (Ditor, Macdonald et al. 2005). From pre-clinical studies it had been shown that exaggerated episodes of AD are elicited with CRD following six weeks of BWSTT (Laird, Carrive et al. 2009).

Hypothesis and Specific Aims

Spinal cord injury-induced cardiac dysfunction is a result of the initial decentralization of the ANS from descending sympatho-excitatory control which is further exacerbated by significant cardiac unloading secondary to decentralization of peripheral vasculature (West, Mills et al. 2012, West, Alyahya et al. 2013), reduced circulating blood volume (Houtman, Oeseburg et al. 2000), and a loss of muscle and respiratory pumps (Faghri and Yount 2002). From clinical studies in the chronic SCI population, FES cycling reverses cardiac atrophy and improve systolic function (Nash, Bilsker et al. 1991). While in pre-clinical studies, PHLC implemented acutely post-injury prevents cardiac structural remodeling, mitigates functional decline, reduces the severity of AD and provides cardio metabolic protective effects (West, Crawford et al. 2014, Squair, West et al. 2016, West, Crawford et al. 2016). However, all of these pre-clinical studies utilized a complete transection model, which is not anatomically representative of the majority of injuries presenting to the clinic. Transection models are most likely used due to the uniformity of the injury and complete paralysis of the hindlimbs.

Although exercise interventions are often prescribed to combat cardiac decline there is no general consensus on how commonly used regimens affect cardiac function following SCI when implemented under the same condition (i.e. time post injury and severity of the injury). Furthermore, implementing these intense interventions can be

laborious and time consuming to complete. Surprisingly, there has been little effort to investigate the role of basal levels of daily (spontaneous) activity on cardiac function/dysfunction. It could very well be the case that manipulating baseline levels of activity, by either increasing or limiting it, could swing CV recovery in a positive or negative direction. In our own lab we have demonstrated that implementing stretching and wheelchair therapies acutely post SCI can negatively influence locomotor recovery, presumably leading to maladaptive plasticity (Caudle, Brown et al. 2011, Caudle, Atkinson et al. 2014). We believe that the ANS will respond similarly to the locomotor and somatosensory components of the CNS and will respond to an appropriately timed therapy aimed at influencing adaptive or maladaptive plasticity.

To date, it had been difficult to separate the relative contributions of the initial ANS decentralization (i.e. the initial injury) from the extreme inactivity to the observed chronic CV function. Furthermore, most of our current understanding of cardiac function comes from load dependent assessments derived from echocardiography and there is little knowledge regarding load-independent measures of LV function following SCI. In addition, no studies have investigated the functional properties of the heart independent of the damaged circuitry.

Thus, we hypothesize that implementing an appropriate exercise intervention acutely post-injury has the potential to protect against maladaptive cardiac remodeling and decline. The proposed experiments will directly address our hypothesis using a clinically relevant rat contusive SCI model subjected to a spectrum of activity conditions. We will investigate changes in anatomical structures that may underlie

cardiovascular remodeling including: descending sympathetic pathways, collagen deposition in cardiac tissue, as well as changes in wall thickness. Using these strategies we will investigate how the intensity of activity/exercise influences cardiovascular function/dysfunction following moderately-severe high-thoracic SCI.

Specific Aim One

Define a clinically relevant model of contusive SCI that results in significant and sustained cardiac deficits. The main goal of this aim is to characterize a model of incomplete SCI that results in sustained cardiac deficits alongside a small degree of residual locomotor function. Several severities of SCI will be administered at the various spinal segments that regulate basal cardiac function. Functional changes will be tracked temporally with echocardiography. This aim will also focus on characterizing cardiac morphological changes (i.e. atrophy and collagen deposition) in response to SCI. A beta one agonist, Dobutamine, will also be employed to investigate changes in response to increased sympathetic stimulation and evaluate cardiac function under stress.

Specific Aim Two

To determine the effects of passive and active exercise interventions on cardiovascular dysfunction following a severe contusive SCI. This aim will compare functional and morphological outcomes induced by passive hind-limb cycling (PHLC) and swim training (SWIM) in animals with a contusive SCI. Echocardiography assessments will be used to track functional changes over time and to make between group comparisons. Changes in cardiac responses to sympathetic stimulation will also be

assessed pharmacologically with the beta agonist, Dobutamine, to determine intrinsic remodeling of the myocardium. Histological changes in serotonergic innervation, adrenergic receptor density, sympathetic preganglionic and postganglionic neuronal changes.

Specific Aim Three

To determine how cardiac structural and functional deficits are influenced by spontaneous activity, post-SCI. Specifically, how SCI induced cardiac changes response to controlled levels of spontaneous activity (activity modified by housing conditions). Echocardiography will be used to correlate functional assessments with changes in sympathetic innervation to myocardial contractile tissue. Echocardiography derived measures will also be correlated with overground stepping patterns and the amount of the overnight activity generated by the animals.

CHAPTER II

GENERAL METHODS

Spinal Cord Injury Surgeries

For SCI surgeries, animals were initially anesthetized with a cocktail of ketamine/xylazine/acepromazine (50/0.024/0.005 mg/kg i.p.). Animals were monitored until they reached surgical depth of anesthetics, which was determined by an absences of a toe pinch and blink reflex. The animals' backs were then shaven and cleaned with 4% Dermachlor Surgical Scrub (Henry Schein Animal Health; Dublin, OH, USA) prior to exposing the SC. A dorsal mid-line incision was then made through the skin, fascia and musculature overlying the C8 to T4 spinal segments for either a thoracic level three (T3) or a thoracic level two (T2) injury.

For T3 injuries, a single level laminectomy was made at the T2 vertebral level to expose to the underlying T3 spinal segment while for T2 injuries a laminectomy was performed on the T1 vertebrae to expose the underlying T2 spinal segment. Using clamps applied to the C8 or T1 and T4 spinous processes, the spine was immobilized and positioned for SCI impact. An NYU impactor was then used to deliver either a mild (12.5g-cm) or moderate-severe (25g-cm) contusion injury (MASCIS Impactor Rutgers University, NJ). Bruising on the SC was verified visually before carefully rinsing the incision with saline and swabbing excess bone and blood away with gauze and/or cotton

applicators. The skin and overlying muscles were sutured back together in layers with silk sutures and antibiotic ointment was applied to incision. Animals were monitored in a temperature-controlled environment and their recovery was observed until a lifting of their head was confirmed. Rats were singly housed for one night following SCI to ensure sutures remained in place and to confirm that each individual rodent was passing stools. Impacted stomachs were massaged and the rodents were given warm baths. Animals were then housed socially with two animals per cage on the same 12-hour light/dark cycle. Post-operatively animals were administered Buprenorphine (0.1 mg/kg, SC) twice a day for three days, gentamycin (Gentamicin sulfate 15 mg/kg SC) once a day for seven days, and Lactated Ringers (5-10mL) for five days and as needed for hydration. Bladders were expressed manually for five to seven days or until spontaneous voiding returned. Drug administration was recorded. The size of the animals bladders and thee quality of the urine was evaluated as either clear, cloudy or bloody Animals' diet was supplemented with DietGel (76A; Portland, Main) and Ensure (Abbot Laboratories, Columbus, OH, USA) when their weight dropped.

Echocardiography

Echocardiography assessments were performed with a commercially available small animal ultrasound system (Vevo 2100 and Vevo 3100 VisualSonics, Toronto, CA and GE Vivid 7, GE Medical, Horten, Norway) and 5.6-14.1 MHz transducers. Rats were anesthetized with isoflurane and maintained at a surgical depth of anesthetic (induction chamber at 5% with 1.5-2 L/min Oxygen flow followed by 2-1.5% with 1.5-2 L/min Oxygen flow). Artificial tears (Henry Schein Animal Health; Dublin, OH, USA) were

placed over the rodents' eyes before the animals' thorax was shaved and cleaned. Rodents were placed in a supine position on an Advanced Physiological Monitoring Unit (VisualSonics; Toronto CA) to maintain core body temperature and monitor HR. The fore-limbs and hind-limbs were secured with surgical tape to conductance strips on the induction pad with a small amount of electrode crème (Indus Instruments, Webster, TX, USA) on each paw to aid conductance. Core temperature was monitored via a rectal probe and maintained at 37-38°C. The HR (250-300 BPM) was monitored continuously with the ECG pad while respirations were monitored via induction plethysmography.

Standard measures of left ventricular structure and function were obtained along the parasternal long axis (PSLAX) and short-axis (SAX) at the mid-ventricular papillary level. Ecogel was applied liberally to the cleaned thorax (Ecogel 100 Imaging Ultrasound Get, Eco-Med Pharmaceuticals Inc. Mississauga, Ontario CA) and was replenished when necessary. Brightness mode (B-mode) images were used for anatomical measurements and movement mode (m-mode) was used to generate systolic function and flow derived indices. In the apical four-chamber view, pulse-wave Doppler was used to estimate early (passive) diastolic filling (E). Results from five cardiac cycles during expiration were averaged together and used for between group and within group (i.e. time-post-SCI) comparisons. Basal echocardiography measurements at the terminal time point were normalized to femur length for between group comparisons.

Dobutamine Stress Echocardiography

Animals were prepped for echocardiography as described above. Once the animals' paws were secured to the conductance strips, the tail was cleaned with dial soap

and heated with Hot Hands (HeatMax Inc. Dalton, GA) to induce dilation of the tail vein. The tail was then placed flat onto a Hot Hand and secured in place with surgical tape such that the tail vein was rotated superiorly and accessible. The vessel was cannulated with a 25-gauge butterfly needle and the needle was secured with surgical tape for the duration drug administration. After prepping the animals for DOB administration, the SAX mid-papillary level was identified manually. The probe was subsequently placed in a stereotactic stand and repositioned for continuous imaging of heart along the SAX. A pre-DOB (0µg) image was then captured for baseline systolic measures. DOB was infused at incrementally increasing dosages (5, 10 and 20µg/kg/min; rates of: 2.10, 4.25 and 8.55 ml/hr) for four minutes each, using an automated perfusion pump (KD Scientific, Holliston, MA). Four minutes of continuous drug infusion have previously been shown to elicit a maximal response at each dose (Plante, Lachance et al. 2005). However, this protocol was later modified for an infusion of 5µg for 5 min and an additional dosage of 30µg/kg/min, since the 20µg dose did not elicit a clear plateau in HR. Within the last 30 seconds of each dose, an M-mode and B-mode image was captured. At the end of the stress test the anesthesia was discontinued and the animals were monitored in a temperature controlled environment until fully recovered.

Pressure Myography

Vessel Preparation

Rats were deeply anesthetized with isoflurane (5% at induction and during maintenance) and surgical depth of anesthetics was confirmed when toe pinch reflex was no longer present. The left and right femoral arteries were isolated from their

neurovascular bundles and the vessel with surrounding muscle was then removed such that the artery itself was the last structure cut. The arteries were isolated from the muscle with the aid of a dissection microscope in physiological saline solution (PSS) containing Ca^{2+} Ringer solution, MOPS, NaH_2PO_4, glucose, pyruvic acid, EDTA, and bovine serum albumin. The arteries were then transferred to a Lucite chamber containing PSS equilibrated with room air. The ends of the arteries were cannulated with a micropipette and secured with nylon suture. PSS was changed every 20 min to refresh substrates required for vasoactivity. Pressure across the vessel was stabilized at 50 mmHg and those arteries that were unable to hold pressure were discarded. Arteries without leaks were warmed, maintained at 37°C and pre-constricted with 10^{-6} serotonin (Ser) (Puzserova, Ilovska et al. 2014). Arteries that were unresponsive to the first two attempts of pre-constriction were also discarded. Albumin was purchased from USB Chemicals (Cleveland, OH). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Endothelium dependent and independent vasodilation

To determine whether SCI alters sensitivity and/or maximal responses to endothelial dependent and independent vasodilation, concentration-response curves to acetylcholine (ACh; dependent) and sodium-nitroprusside (SNP; independent) were generated. Changes in diameter were measured in response to cumulative additions of ACh and SNP (1×10^{-4} M – 6×10^{-9} M; 3- and 2-minute stages respectively) to the vessel bath.

Pressure Volume Analysis

Rats were anesthetized with isoflurane (5% at induction with a 1-2 L/min oxygen flow) and held under surgical depths of anesthetics (2.5-2% maintenance). Aniamls' core body temperature was maintained at 37°C and a three-lead ECG was used to monitor HR through LabChart8 Software. Animals' necks were shaved, cleaned and the right common carotid artery was exposed via a midline neck incision. The pre-tracheal muscles were retracted to provide a clear window to the artery and three silk suture ligatures were placed loosely around it. The most rostral ligature was then tied off while the most caudal ligature was elevated to temporarily occlude the artery. A small incision was made in the artery with a bent tip needle and a 2-Fr microtip pressure-conductance catheter (SPR-869, Millar Instruments, Houston, TX) was inserted and secured in place with the final (middle) suture. The caudal suture was then released and the conductance catheter rested in the right common carotid for five minutes to collect beat-by-beat blood pressure for resting mean arterial pressure (MAP) and systemic vascular resistance (SVR) calculations. The catheter was advanced into the LV under pressure guidance and after optimizing the pressure-volume signals, 10 minutes of resting data were recorded at 1000Hz (Powerlab, ADInstruments, Colorado Springs, CO USA) and visualized with commercially available software (LabChart8.1 Plus Software, AD instruments).

Using specialized PV analysis software (PVAN, Millar Instruments), the end-systolic pressure (ESP), end-diastolic pressure (EDP), the maximal slope of LV systolic pressure increment (dP/dt_{max}) and diastolic pressure decrement (dP/dt_{min}), ejection fraction (EF), and stroke work (SW) were computed and calculated off-line. Stroke volume (SV) and cardiac output (CO) were calculated and corrected according to PVAN

software calibrations. To account for the influence of body mass differences, CO and SW were also normalized to body mass (cardiac index, CI and SW index, SWI).

After acquiring basal PV measurements, DOB was infused in the same step-wise manner as described above, in progressively increasing concentrations. Pressure and volumetric responses to DOB were also analyzed off-line with specialized PV analysis software. Following DOB infusion and data collection, the animals recovered for 10 min before assessing load-independent metrics. The IVC was approached through a mid-line abdominal incision and occluded with a cotton applicator three consecutive times to progressively reduce preload and allow the calculation of load-independent measures of LV contractility including the end-systolic pressure volume relationship (Ees), preload recruitable stroke work (PRSW), and the slope of the dP/d*t*max end-diastolic volume relationship (dP/d*t*max-EDV). Calibration was then performed in accordance with procedures that have been well described previously (Pacher, Nagayama et al. 2008). Briefly, the pressure and conductance were calibrated using the internal standard signal calibration. Saline (hypertonic, 50µl) was injected into the already exposed IVC to calculate the conductance volume and correct for cardiac mass volume interference during PV collection. Finally, the conductance signal was calibrated to absolute volumes using a cuvette calibration wells filled with fresh, heparinized, warm blood. All data analysis of PV relationships was conducted in Labchart8 (ADInstruments, Colorado Springs, CO USA). Animals were then euthanized with an overdosed with isoflurane and the heart was excised.

Overnight Activity

Animals' cages were placed on commercially available wire racks fitted with custom-made camera mounts (Fig.5A). Cameras (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) were fitted with specialized lenses to capture a wide view (Fujinon DF6HA-1B; 1:1.2/6mm Fixed Focal Lens Fujifilm, Japan) and mounted above the cages (Fig.5B,C). Two standard cages, one large cage or two tiny cages can be recorded using one camera mount. Two infrared lights (CM-IR30 IR Illuminator; C&M Vision Technologies Inc, Houston, TX) were placed on either side of the mounted camera and their brightness was adjusted such that there were no sections of the cage obscured from a light glare. The cameras were connected via an Ethernet cord to a computer tower programmed with a custom-made Labview program (2014 64-bit; National Instruments, Houston, TX). One minute was recorded every ten minutes for 12 hours (6 min per hour). Videos were captured at a frame rate of 4Hz (4 frames per second). The distance animals traveled (or covered) overnight was analyzed off line with MaxTRAQ (version 2.4, Innovision systems; Columbiaville, MI, USA) and used for between group comparisons (Fig6).

Open field test

The basal anxiety levels of rodents can be quantified with the previously defined open field test, which compares the amount of time animals stay at the edges of a grid marked arena (Bignami 1996). We constructed an area with four black Plexiglas pieces fitted together with cutout slats. The bottomless area measured 70x70x22cm (width x length x height). The arena was placed on standard black laboratory countertop and the base of the arena was divided into 16 squares measuring 17.5x17.5 cm with black

electrical tape (Fig.7). All materials used to construct the open field area were black so that movement of the white rodents within the space was easily followed. The tests were recorded using Basler cameras (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) and saved into a custom-made LabView program (2014 64-bit; National Instruments, Houston, TX). At the start of the assessment, one rat was placed directly in the center of the arena and their movements were recorded at 8Hz for five minutes. The arena was thoroughly cleaned with 70 percent ethanol (EtOH) before introducing another animal for assessment. The open field test was conducted in a quiet room and by the same handler for each time point. The amount of time animals spent in the perimeter grids was compared to the time they spent in the middle grids. The number of times the animals crossed the middle grids was also quantified off-line.

Exercise Training

Passive hind-limb cycling

Passive hind-limb cycling was accomplished using a custom-made ergometer as previously described (Fig.8). The training was completed 5 days a week for 30 min each day. Rats were horizontally suspended in a leather sling with two holes cut for their hind-limbs. Their hind paws were secured to the bikes pedals with gauze (to prevent skin abrasions) and Parafilm. Rats were cycled at a frequency of 0.5 Hz and monitored continuously. Rats were also provided cheerios and fruit loops during their cycling sessions. After completing a cycle training session the animals' ankles were inspected for abrasions and the animals were returned to their cages.

Swim training

Swimming was completed 5 days per week, for 30min of exercise per day. Swimming was broken down into sessions such that animals swam six, five minute intervals with breaks in-between the sessions. Animals swam in a custom-made lap pool (Fig.9; 60" length x18" deep x 7" wide) constructed from Plexiglas. The pools were filled with warm water until the majority of the exit ramp was covered, roughly 6 inches from the top of the pool. Pool water was maintained at 32°C for the duration of the swimming sessions and was warmed when needed. To begin each swimming session, animals were placed at one end of the pool and encouraged to swim with aid, by either tail or slight trunk support, to a padded ramp at the other end. Once the animal reached the ramp they were picked up and re-set to the opposite end of the pool to start another swim lap. During the swimming sessions, the pool was cleaned of any fecal matter with a fish net. Once the animals finished swimming, the pools were drained and thoroughly cleaned with concentrated detergent and 70 percent EtOH.

Tissue Processing

Animal termination and tissue collection

At the end of each experiment, animals were sacrificed with an overdose of ketamine (87.5mg/kg). A thoracotomy was then performed via an abdominal approach where the abdominal muscles were incised at the midline and the incisions were extended laterally following the inferior border of the diaphragm/costal margin. The diaphragm was carefully cut open at the central tendon, just to the left (anatomical right) of where the apex of the heart is and the diaphragm was divided from its attachments to the costal

margin. The thoracic cage was reflected superiorly by making bilateral cuts through the ribs along the midaxiallry line extending to the axilla. Using hemostats, the thoracic cage was held open and the heart was cleaned of fat and remnant thymus. The ascending aorta was carefully dissected and fascial attachments to the pulmonary trunk were carefully released. One tine of a hemostat was slipped under the ascending aorta and the other superior to it. An 18 gauge needle attached to a perfusion pump was threaded into the aorta and clamped into place with the hemostat positioned around the aorta. Once the bevel was visualized in the ascending and arch of the aorta, the perfusion pump was started. A small cut was made in the right atria to allow blood perfusion to occur. Once 500mL of perfusion fluid was pumped and the animal limbs were confirmed to be pale and devoid of blood the heart was excised, cleaned of excess vessels and fat and weighed with an analytical scale. The hearts were placed in 4% paraformaldehyde (PFA) for 24 hours. Hearts were transferred to 30% sucrose for 24 hrs and then placed in optimal tissue cryoprotecting media. The spinal column was cleaned of the deep back muscles and rib attachments. The column was cut at the base of the skull and at the iliac crest and placed in 4%PFA for 3 days. The spinal cord was removed from the column and the lesion site was verified by counting spinal cord roots. The cord was placed into 30% sucrose and the epicenter was blocked after 1 day of cryoprotecting.

Eriochrome Cyanin

Spinal Cords (SC) were sectioned through the epicenter at 30µm. Sections were allowed to air dry for 30min before storing at 4°C overnight. The following day, SC

sections were warmed for 20mins and processed for spared white matter using Eriochrome Cyanin (EC) stain as described previously (Smith, Burke et al. 2006).

Masson's Trichrome

Hearts were sectioned at 10μm thick at the midventricular level. Sections were allowed to dry for 30 minutes before storing them in -20 °C overnight. Sections were thawed for 20 minutes at 37°C. Slides were immersed in Bouin's solution (HT10132; Sigma-Aldrich; St. Louis, MO, USA) overnight for 18 hours. Slides were rinsed in cold tap water for 30 min the following day. A working solution was made from phosphotunic : phosphomolybolic : distilled water (1:1:2; HT15, Sigma-Aldrich; St. Louis, MO, USA) the day of staining during the morning rinse and left over was discarded. Slides were dried and placed in Biebrich (HT151, Sigma-Aldrich; St. Louis, MO, USA) solution for 8 min and subsequently moved to distilled water for 10 min. Slides were then laid flat on a paper towel and the working solution was added dropwise over each section and incubated for 5 min. The solution was discarded and Analine Blue (B8563, Sigma-Aldrich; St. Louis, MO, USA) was added in the same dropwiase manner for 5 min. Sections were then transferred to 1% Acetic Acid for 2 min to stabilize the blue stain. Slides were then sequential dehydrated (70%, 95%, 100%) and allowed to dry for 10 minutes. Slides were dipped into xylene and applying a coverslip fixed in place with paramount.

Hematoxylin & Eosin

Slides were thawed for 20 minutes at 37°C on a slide warmer. They were then dehydrated for 2 minutes in 95 percent EtOH and then transferred to Hematoxylin

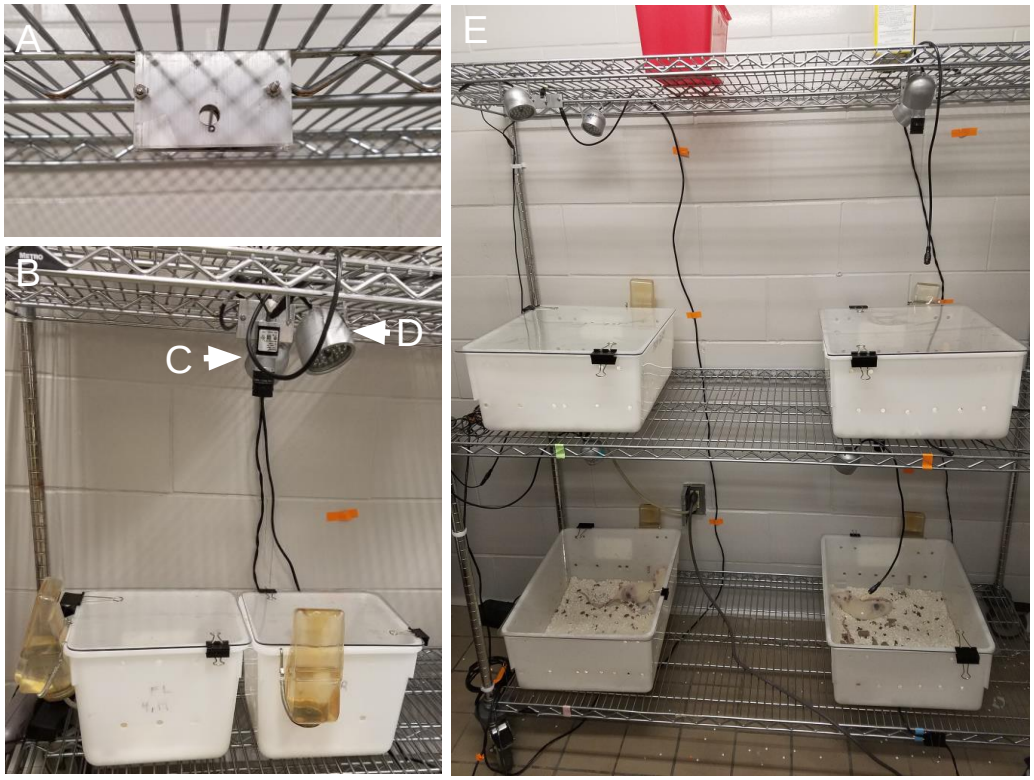
(CAT#245-264, Fischer Scientific, Kalamazoo, MI, USA) for 2 min. Hematoxylin was washed off with two water dips in distilled water and subsequently air dried fro 10 min. Sections were then stained with Eosin (CAT# 23-314631, Fischer Scientific, Kalamazoo, MI, USA) for 2 min, dehydrated with 70 and 100 percent EtOH for 1 min each. Sections were then air dried for 10 min, dipped in xylene and coverslipped with paramount.

Immunohistochemistry

Heart sections were thawed for 20 minutes at 37°C and pap-penned, followed by re-hydration in 0.1M phosphate buffered saline (PBS) for 10 minutes. Sections were then incubated with 10% normal donkey serum for 30 minutes. After removal of normal donkey serum, sections were incubated in the primary antibody rabbit anti-tyrosin hydroxylase (TH, 1:500; Millipore, ab152) prepared in 0.1M PBS-Triton (0.3%) overnight at room temperature in black humidity box, on a stir pad. The next morning three 10 minute 0.1M PBS washes were done to remove the primary antibodies, after which they were incubated with the secondary antibody Texas Red Jackson, donkey anti rabbit for two hours. Sections were then cover slipped using fluoromount and stored at 4°C. Images were acquired with a Nikon 400 fluorescence microscope and images were analyzed with ImageJ (National Institute of Health, Bethesda MD).

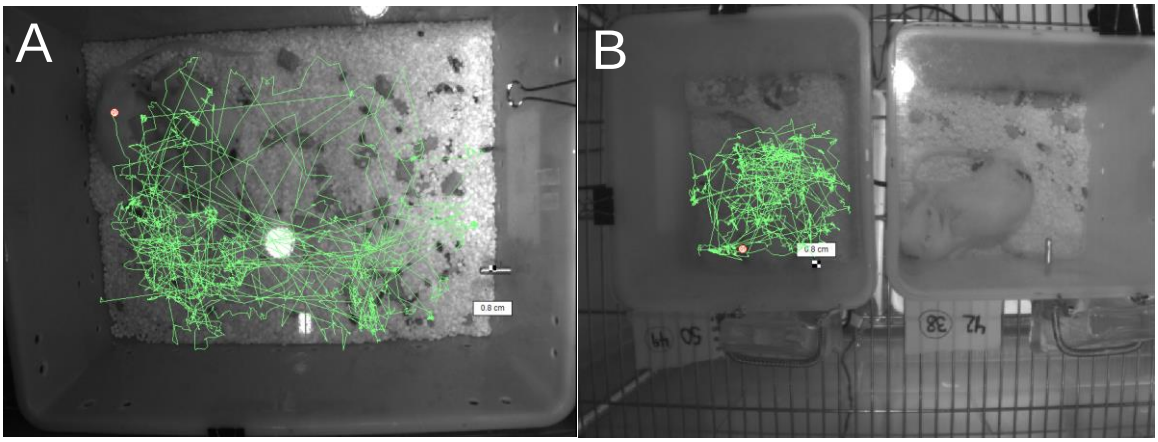
Spinal cord sections were thawed for 20 minutes at 37°C and pap-penned, followed by re-hydration in 0.1M PBS for 10 minutes. Sections were then incubated with 10% normal donkey serum for 20 minutes. After removal of normal donkey serum, sections were incubated in primary antibody prepared in 0.1M PBS-Triton (0.1%) overnight at room temperature in black humidity box, on a stir pad. The next morning

three 10 minute 0.1M PBS washes were done to remove the primary antibodies, after which they were incubated with secondary antibodies for two hours. The following primary antibodies were used: goat Anti-Choline Acetyltransferase (ChAT, 1:500; Millipore, ab144P); anti Neuronal Nuclei (NeuN, 1:200; Chemicon, MAB377); and Hoechst (1:1000). Secondary antibodies were used: Alexa Fluor 594 Jackson donkey anti goat (CHAT); Texas Red Jackson donkey anti goat (CHAT); FITC Jackson donkey anti mouse (NeuN). Sections were then cover slipped using fluoromount and stored at 4°C. Images were captured with an inverted fluorescent microscope (Nikon Eclipse Ti) and imaging program. Images were analyzed with ImageJ (National Institute of Health, Bethesda MD).



<u>Figure 5</u>. Overnight set-up.

Example cages are from the tiny cages tiny vs. large cage study (chapter IV). A, custom made mount for camera stand. B, Overnight set up with tiny cages, two tiny cages captured under one camera. C, Wide lens camera on custom made mount. D, Infrared lights. E, wire racks with large cages, only one large cage can be captured per camera.



<u>Figure 6.</u> Representative images of overnight output generated by MaxTRAQ tracking software.
A. The overnight activity of a large caged animal and B. overnight activity of a tiny caged animal.

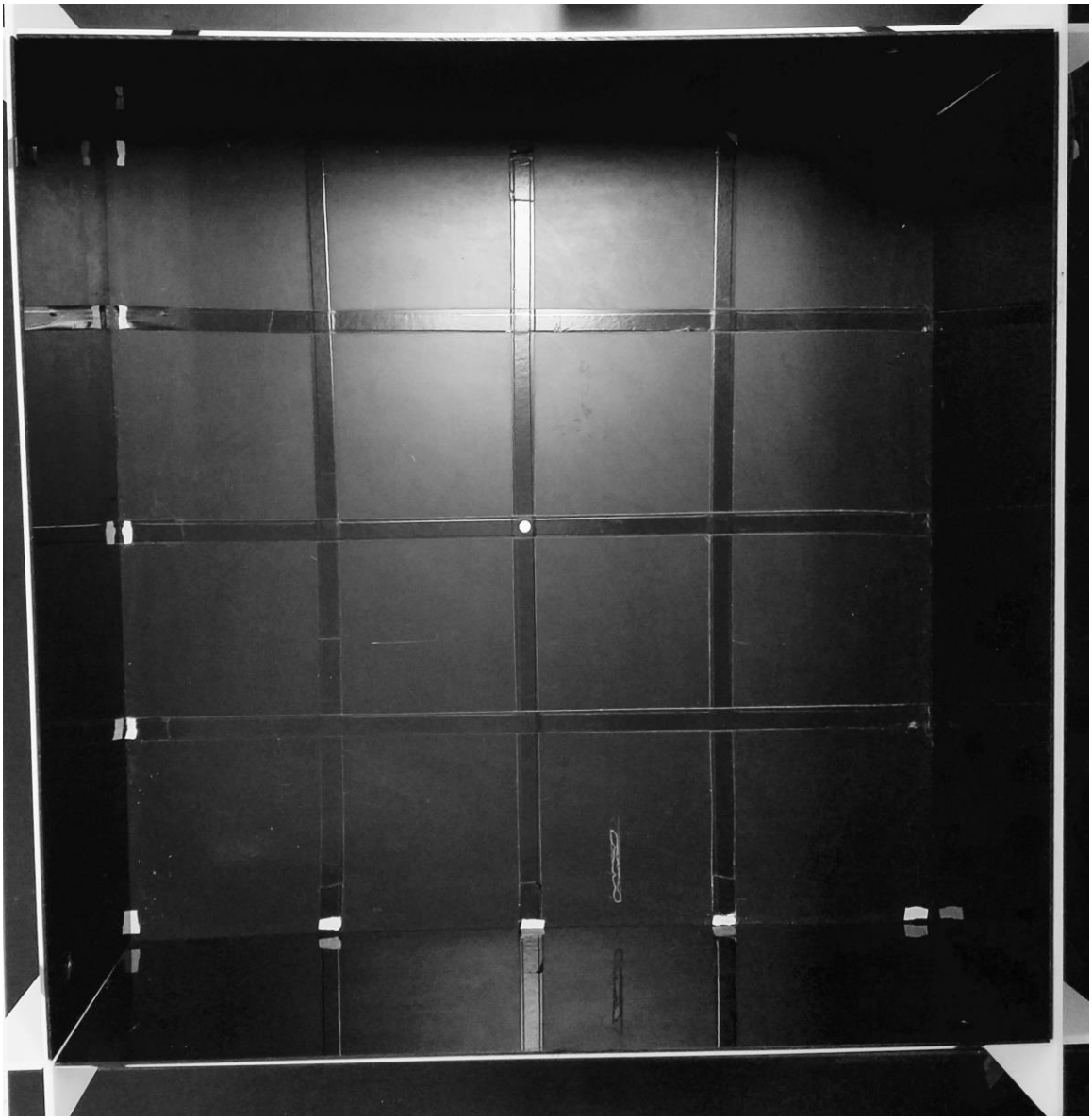
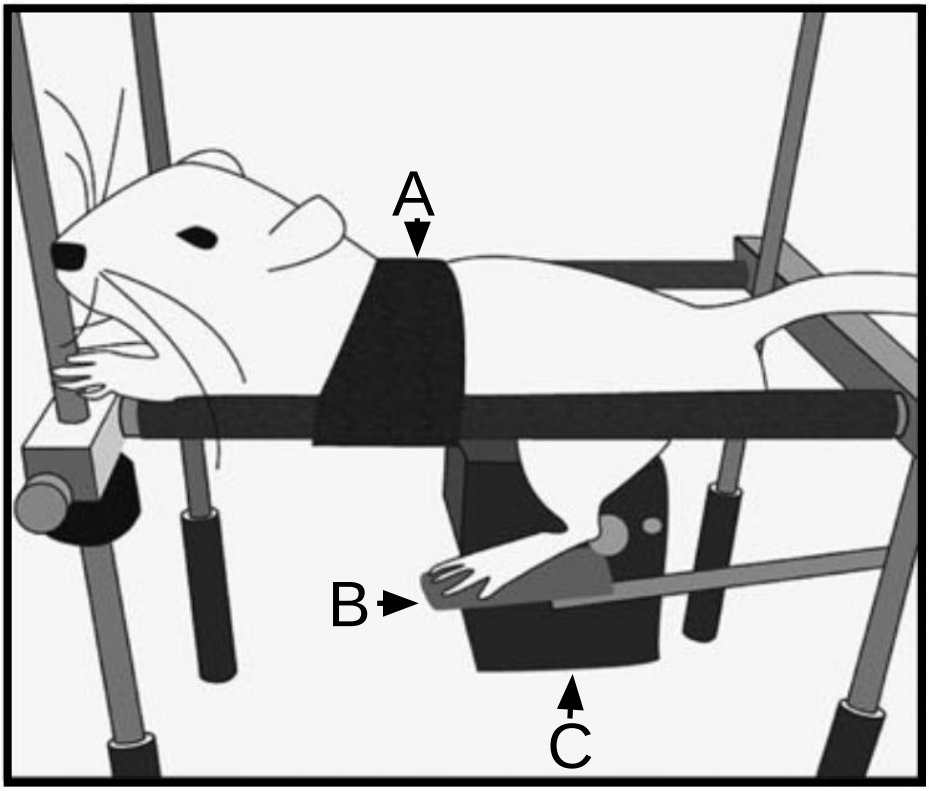


Figure 7. The open-field arena constructed from plexiglass.

Center marker indicates where the animals were to be placed at the beginning of each assessment. Each grid measures 17.5x17.5 cm. The arena is 70x70 cm width and length and 22 cm tall so that the animals could not jump out. A camera was suspended above the arena in a level plane to record the movement of the animals.



<u>Figure 8</u>. Schematic of passive hind-limb cycling custom made ergometer bikes.

A. Leather harness with two holes cut out for the hind-limbs to pass through. B. Pedals for the rodents' feet. C. The bike motor.

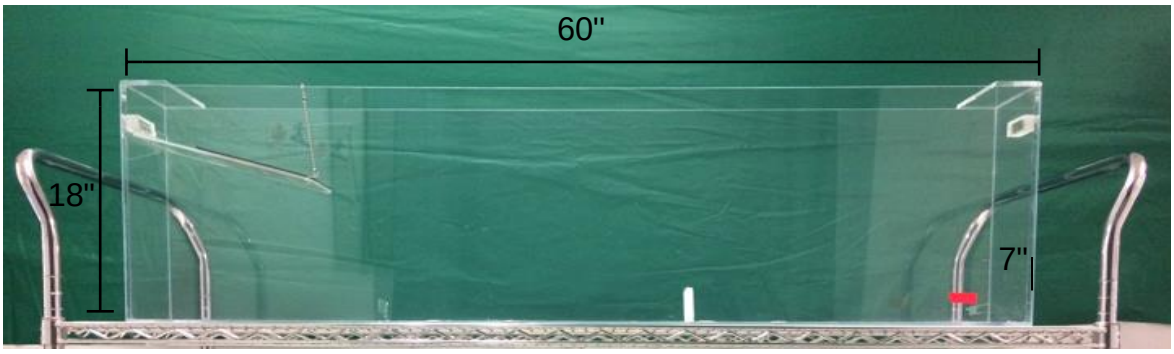


Figure 9. Custom-made swimming tank.

The tank is constructed with clear Plexiglas and sealed with specialized Plexiglas adherent. The sides are fitted with handles also attached with Plexiglas glue and the middle is stabilized with a bracket for extra support when the pool is filled with water. At the bottom there is hole cut to drain the pool of water following swim session and is plugged with a rubber stopper during swim sessions.

CHAPTER III

CHARACTERIZING A CLINICALLY RELEVANT CONTUSION MODEL OF SPINAL CORD INJURY WITH PERSISTENT CARDIOVASCULAR DEFICITS

Introduction

In addition to the devastating sensory and locomotor paralysis, individuals with spinal cord injury (SCI) face a myriad of secondary complications. Cardiovascular (CV) consequences induced by the SCI are some of the most deleterious. The magnitude of CV abnormalities is heavily depended on the level of injury, where individuals sustaining cervical and high thoracic SCI suffer from systolic dysfunction, constant resting hypotension, bradycardia, orthostatic hypotension and episodic hypertension (autonomic dysreflexia). Autonomic dysreflexia (AD) elicited by either noxious or non-noxious stimulus below the level of the lesion is potentially life threatening (Eltorai, Kim et al. 1992, Pan, Wang et al. 2005, Ho and Krassioukov 2010). Furthermore, the autonomic completeness of the injury, as determined by the sympathetic skin response (SSR), indicates the magnitude of chronic CV abnormalities such that the greatest CV impairments are observed in conjunction with a lack of positive SSR (Ravensbergen, Walsh et al. 2012, West, Bellantoni et al. 2013). Autonomically complete cervical injuries also display the greatest impairments in systolic function and resting heart rate (HR) compared to injuries at the thoracic and lumbar levels. Therefore there is now

general consensus that the degree of chronic cardiovascular deficits is correlated to the level *and* severity of injury (West, Mills et al. 2012, Currie, West et al. 2014, West, Gee et al. 2015).

Despite the evident influence of the injury completeness to the manifestation of chronic CV deficits, the majority of animal studies investigating CV function following SCI utilize full transection models. Both the temporal development of systolic dysfunction and blood pressure (BP) dysregulation, which manifest by six weeks post-SCI, have been characterized with a thoracic level three (T3) transection model (West, Crawford et al. 2014, West, Popok et al. 2015). Transections at the thoracic five (T5) level have revealed altered electrophysiology, increased risk of arrhythmias (Lujan and DiCarlo 2007), increased sympathetic fiber density to the left ventricle free wall (Lujan, Palani et al. 2010, Lujan, Janbaih et al. 2012), and a reduced resting mean arterial pressure (MAP) (Laird, Carrive et al. 2006). It is important to note that with a T5 injury, sympathetic innervation to the heart is spared (Teasell, Arnold et al. 2000, Krassioukov 2009) which contributes to the specific cardiac manifestations observed. Transection models are beneficial due to the complete disruption of ascending and descending circuitry and subsequent development of cardiac impairments; however, a large portion of SCI cases present with some degree of spared circuitry.

Contusion SCI is an ideal model for investigating cardiac consequences post injury due to the varying degrees of spared circuitry from the impact. A wide range of contusion models, differing in both the lesion level and severity, are already established for investigating locomotor consequences of SCI (Magnuson, Trinder et al. 1999, Mayorov, Adams et al. 2001). Traditionally, a critical component of these models is that

the degree of white matter disruption is proportional to the locomotor recovery (Basso, Beattie et al. 1995, Schucht, Raineteau et al. 2002). However, tonic and phasic cardiovascular function is regulated by the autonomic nervous system (sympathetic and parasympathetic; ANS) and the pathway by which the ANS reaches target organs differs from the surpaspinal centers influencing locomotor function. It has not been demonstrated that the amount of white matter spared following injury correlates with the chronic cardiovascular function in contusion rodent model. However, in the clinical setting it has been shown that the integrity of sympathetic pathways (autonomic completeness of injury) is correlated to indices of exercise performance (West, Romer et al. 2013).

Characterizing a contusion model that elicits persistent cardiovascular dysfunction is incredibly important to further our knowledge of cardiovascular complications in incomplete SCI. To our knowledge this has yet to be accomplished. Therefore the purpose of this study was to characterize a contusive SCI that regains some degree of overground stepping in addition to substantial and persistent cardiac dysfunction.

Methods

Experimental design

Initial experiments were performed on six female Sprague Dawley (SD) rats. Animals sustained a mild 12.5g-cm, T3 (T3-MILD, n=6) SCI. Cardiac function was assessed with *in vivo* echocardiography prior to injury and bi-weekly following SCI. Open field locomotor assessment (BBB) was performed weekly. Since we found sustained (i.e. a plateau) cardiac function at six weeks post-SCI, we conducted a follow-

up experiment to evaluate cardiac function following a more severe and higher-level lesion injury. Twenty-two SD female rats were divided into either a moderate 25g-cm thoracic level 2 (T2-MOD) or a T3 (T3-MOD) SCI group. Three animals were lost following surgery leaving eleven animals (n=11) in the T2-MOD group and eight (n=8) animals in the T3-MOD group. Cardiac function was assessed with *in vivo* echocardiography prior to injury and bi-weekly following SCI. Open field locomotor assessment (BBB) was performed weekly Since we observed sustained deficits in resting cardiac measures in the T2-MOD group, we harvested vessels from these animals (n=10) at six weeks post-injury for pressure myography assessments of the femoral arteries. Six female, age-matched, SD rats were used as controls (CON) for vessel response curves and histological assessments.

Spinal Cord Injury Surgery

On the day of surgery, animals were anesthetized with a ketamine/xylazine/acepromazine cocktail (50/0.024/0.005 mg/kg) and a dorsal mid-line incision was made through the skin and musculature overlaying the C8 to T4 spinal segments. A laminectomy was performed at either the T2 vertebral level to expose the underlying T3 spinal cord and at the T1 vertebral level to expose the T1 spinal segment. Either a moderately-severe injury (25g-cm) or mild (12.5g-cm) injury was administered with a MASCIS Impactor (Rutgers University, NJ). All animals were given Buprenorphine (0.1 mg/kg, SC) twice a day for three days, gentamycin (Gentamicin sulfate 15 mg/kg SC) once a day for seven days, and 5mL of Lactated Ringers for five days and as needed for hydration post-SCI. Bladders were expressed manually for five

days or until spontaneous voiding returned. After recovery, animals were housed socially (two per cage) on the same 12-hour light/dark cycle in a temperature-controlled environment.

In vivo echocardiography

Echocardiography was performed with a commercially available small animal imaging system (Visualsonics Vevo 2100; Toronto, ON, Canada) and probe (13-24Mhz, Visualsonics). Animals were anesthetized with isoflurane (5% at induction chamber; followed by 1.5-2% maintenance), placed in supine position and secured to a rodent echo platform (Visualsonics; Toronto, ON, Canada) with surgical tape. Body temperature was maintained at 36-37.5°C and ventilation was monitored by induction plethysmography. Images were captured along the parasternal short axis to generate standard measures of left ventricle structure and function. Pulse wave Doppler was used to estimate early and late filling during diastole. Results from ten cardiac cycles during expiration were averaged together for between and within group comparisons.

Pressure myography

i. Vessel Preparation

Rats were deeply anesthetized with isoflurane (5% at induction and during maintenance). The left and right femoral arteries were isolated from their neurovascular bundles and the vessel with surrounding muscle attached was then removed. Both arteries were harvested in the event that one would not hold pressure or pre-constrict. The arteries were then removed from the muscle with the aid of a dissection microscope (Olympus

SVH10) in physiological saline solution (PSS) containing 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 ml BSA, pH 7.4. The arteries were transferred to a Lucite chamber containing PSS equilibrated with room air. The ends of the arteries were cannulated with a micropipette and secured with nylon suture. The chamber was then placed on an inverted microscope (Olympus IX70) equipped with a video camera and micrometer (Panasonic BP310; Texas A&M Cardiovascular Research Institute) to measure intraluminal diameter. The arteries were pressurized to 50mmHg with two hydrostatic columns and those unable to hold pressure were discarded. Arteries without leaks were warmed, maintained at 37°C and pre-constricted with 10^{-6} serotonin (Ser) (Puzserova, Ilovska et al. 2014). Arteries that were unresponsive to the first two attempts of pre-constriction were discarded. Albumin was purchased from USB Chemicals (Cleveland, OH). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

ii. Endothelium dependent and independent vasodilation

To determine whether SCI alters sensitivity and/or maximal responses to endothelial dependent and independent vasodilation, concentration-response curves to acetylcholine (ACh; dependent) and sodium-nitroprusside (SNP; independent) were generated. Changes in diameter were measured in response to cumulative additions of ACh and SNP (1×10^{-4} M – 6×10^{-9} M; 3- and 2-minute stages respectively) to the vessel bath.

Histology and Immunohistochemistry

Animals were perfused via an ascending aorta cannulation with phosphate buffered saline (PBS). The heart and spinal column were immediately harvested following perfusion. The heart was cleaned of excess tissue (fat and the great vessels), weighed and fixed in 4% paraformaldehyde for 24hours. It was then cryoprotected, divided into three sections (base, mid-ventricular and apex) and blocked in optimal tissue cutting (OTC) media. The mid-ventricular section was cut at 10μm and processed for the presence of collagen and the density of sympathetic innervation.

Spinal cords (SC) remained in the columns and were fixed in 4% paraformaldehyde for three days. Spinal cords were then removed from the column and cryoprotected. The epicenter was visually identified, isolated and blocked from cervical level eight to one mm caudal the epicenter in optimal cutting temperature (OTC) compound. The SC was then sectioned at 30μm. White matter spared was quantified with eriochrome cyanine as previously described (Hadi, Zhang et al. 2000, Magnuson, Lovett et al. 2005).

Collagen content in the left ventricle was quantified with Masson's Trichrome stain by threshold discrimination with ImageJ software. Specifically, with a Nixon (E400) microscope, ten images from each animal were captured from sections at least 70μm apart with the 20x objective. Collagen was identified by hue, brightness and saturation discrimination and quantified as a percent of the total area of the image captured (area of the collagen/area of field of vision X 100%; $40\mu m^2$) and compared between groups.

The left ventricular free wall was also processed for sympathetic innervation

density. Slides were air-dried, washed in PBS (2x10min) and blocked for 20 minutes in 10% normal donkey serum PBS-triton (PBS-Tx). Slides were incubated overnight at room temperature with rabbit anti-tyrosine hydroxylase (1:500, Milipore AB125) in 0.3%PBS-Tx. After being rinsed in PBS (2x10min,) sections were incubated in Alexa Fluora 594 donkey anti-rabbit for two hours. Sections were rinsed 2x10min with PBS. Images of the left ventricle free wall (LVFW) were captured with Nixon bright-field microscope under consistent settings. Eight sections, at least 70μm apart, from each animal were captured at 40x and analyzed for sympathetic nerve fiber density. In ImageJ, images were 2048x2048 pixels (4,194,304 total pixels) and pixels were scaled to mm such that the total area of the image was 0.0961mm^2. Threshold discrimination was used to eliminate ventricular tissue and quantify the amount of sympathetic nerve fibers present in the myocardium. Sympathetic nerve fibers were quantified as the percent of total area; specifically, area of tyrosine hydroxylase positive fibers was divided by the total area of the image (Lujan, Janbaih et al. 2012).

Dose response data analyses

Dose response curves and vessel characteristics are represented as means ± SEM

$$\text{Spontaneous Tone (\%)} = \{(D_M - D_T)/D_M\} \times 100$$

where D_M is the maximal diameter recorded at 50 mmHg and D_T is the steady-state baseline diameter recorded at the same pressure. Constriction to ACh and SNP was expressed by the following equation:

$$\text{Constriction(\%)} = \{(D_b - D_s)/D_b\} \times 100$$

where D_b is the baseline diameter immediately prior to addition of the first dose of vasoconstrictor agonist, and D_s is the steady state diameter measured after addition of each dose. Passive myogenic response curve was generated using normalized diameter for every pressure point according to the formula as following:

$$\text{Normalized diameter} = D_s/D_{max}$$

where D_{max} is the maximal inner diameter recorded at a pressure of 45 mmHg under Ca^{2+}-free conditions and D_s is the steady diameter after each pressure change. The concentration that produced 50% of the maximal vasoconstriction to the agonist was designated as the EC_{50}.

Statistical Analysis

Due to small sample sizes and high variability, non-parametric analyses Wilcoxin signed ranks were used to determine significance within groups for echocardiography assessments. Data are therefore represented as group means. Significance was set at $P<0.5$ for all assessments.

Dose responses curves of the femoral artery and vessel characteristics were analyzed using two way repeated measures analyses of variance (RM ANOVA) with a factor for group and a factor for dose to determine significant main effects and *post hoc* t-tests were conducted with Bonferonni if a significant main effect was detected. Vessel data are represented as means ± standard error unless noted otherwise.

General linear model ANOVA was used to compare terminal echocardiography assessments. Echocardiography data are represented as means ± standard deviations (SD)

Results

Resting cardiac function

The T3-MILD injury did not result in significantly sustained cardiac dysfunction or changes in heart rate (HR) at six weeks post-SCI (data not shown). Although there was an initial reduction in global, flow-derived indices (stroke volume, ejection fraction, and cardiac output) at one week post-SCI, by six weeks these metrics returned to pre-injury levels. In addition, the left ventricle internal diameter (LVIDd), posterior wall thickness (PWT) and relative wall thickness (RWT) also remained unchanged (Table 1).

Due to the lack of dysfunction at six weeks post-SCI with the T3-MILD injury, two follow up experiments were performed. Increasing the severity of the injury (i.e T3-MOD group) at the same spinal segment also did not result in HR changes, chamber reductions or significantly diminished systolic function compared to pre-injury levels (Fig.10A-F). Moving rostral one spinal segment, to the T2 level, and utilizing the moderate/severe injury did indeed induce systolic cardiac dysfunction. Heart rate was not significantly reduced at early time points but was diminished by six weeks post-SCI (Fig.10A; $P<.05$). One week following SCI, there was a significant reduction in LVIDd and subsequently end diastolic volume (EDV). Also by one week post-SCI, T2-MOD injuries displayed attenuated stroke volume (SV), cardiac output (CO) and ejection fraction (EF; Fig.10D-F; all $P<.05$). By six weeks post-SCI, in the T2-MOD group reductions in LVIDd and EDV were similar to baseline (Fig.10B&C both $P=.093$). However, diminished systolic function did remain present (Fig.10D-F all $P<.05$).

Locomotor function

The locomotor open field test (BBB) revealed a severe drop in function one week post-SCI in all injury groups. Between group comparisons demonstrated that the T3 and T2-MOD injuries had a greater impairment in overground stepping compared to T3-MILD injuries at all time points following SCI (Fig.11A). The T3-MILD injury group began plantar placing by one week post-SCI although they never regained locomotor function comparable to pre-injury levels (Fig.11A). By six weeks post-SCI the T3-MILD group had an average BBB of 17. Animals with T3-MOD began plantar placing with occasional weight supported stepping (BBB=10) by week three while the T2-MOD injury reached a BBB of 10 by week four (Basso, Beattie et al. 1995). By six weeks post SCI the T3-MOD group displayed an average BBB or 11 while the T2-MOD had an average BBB of 10 and thus never regained function comparable to pre-injury levels.

The percent of spared white matter (WM) assessed as darkly (blue) stained compact white matter revealed that there was significantly more WM spared with the T3-MILD injury compared to both moderate/severe injuries (Fig.11C-F). There was no significant difference of WM spared between the moderate/severe injury groups. A range of 23.87 to 12.5 percent WM spared was generated from the T3-MILD group (Fig.11D average 17.41 ± 3.7). The WM spared in the T3-MOD group ranged from 0.68 to 8.11 (Fig.11E average 5.79 ± 3.1). The T2-MOD injury resulted in a range of 3.56 to 9.39 percent spared WM (Fig.11F average 6.5 ± 1.92).

Cardiac histology and immunohistochemistry

The amount of collagen present in the left ventricular free wall, assessed as a percent of total area, was increased in the T3-MILD group compared to CON at six

weeks post-SCI (Fig.12A P<.001). Interestingly, there was no increase in collagen content compared to CON in the T3-MOD group (P=0.925) although there was an increase collagen deposition with the T2-MOD group (P<.05). Furthermore, there was more collagen deposition in the T3-MILD group compared to the T3 and T2-MOD groups (Fig.12A P<.01).

Contrary to previous reports, sympathetic nerve fiber density to the left ventricle free wall was not significantly increased in any of the SCI groups. One animal from the T2-MOD group was eliminated from tyrosine hydroxylase (TH) staining because the tissue was fixed improperly for immunohistochemistry analysis.

Femoral artery responses to ACh and SNP and vessel characteristics

Despite significant impairments in cardiac function following T2-MOD SCI, there appeared to be no significant endothelial dysfunction in vessels below the lesion. One animal was excluded from analysis due to damage to the vessel during removal and subsequent failure of the vessel to hold basal tone with Ser. In the presence of L-NAME, vasodilation was abolished in both T2-MOD injured and CON animals. Responses to SNP were similar between the CON and T2 SCI group (Fig.13). Vessel characteristics are outlined in Table 2. Although SCI did not result in statistically significant alterations of endothelial independent or dependent vasodilation (Fig.13A-C), there was a pattern of increased responsiveness (as assessed with the EC_{50}) to lower concentrations of ACh demonstrated by the leftward shift in the dose response curve.

Discussion

We first demonstrate the cardiac effects of various contusion injuries, differing in both the level and severity of injury, which regain some degree of overground stepping. Specifically, the mild injury (12.5g-cm) did not induce cardiac decline and only the most severe injury at the highest levels (T2-MOD) produced substantial decline. We then reveal that there are only modest changes in vascular responses to either endothelial dependent or independent vasodilation in vessels below the level of the lesion following a T2-MOD injury. We corroborate previous finding of increased collagen deposition in the left ventricle (Lujan, Janbaih et al. 2014) following SCI but did not find evidence of increased sympathetic arborization in the left ventricle free wall (Lujan, Palani et al. 2010).

First we revealed that mild injuries (12.5g-cm) resulted in reduced systolic function acutely post-SCI (one week) but by six weeks post-injury the cardiac function recovered to pre-injury levels. The acute reduction in cardiac function is most likely due to neurogenic shock. Immediately following injury there are marked reductions in blood pressure and HR that ultimately result in diminished cardiac function. Although we did not test this posit directly, it is well documented that neurogenic shock is common in cervical and high thoracic injuries (Bilello, Davis et al. 2003, Krassioukov and Claydon 2006) and is therefore likely present following all high thoracic injuries utilized within this study. The reversal of cardiac function to pre-injury levels in the T3-MILD group is most likely due to the recovery of weight supported stepping (WM spared average 17.4 percent ± 3.7) and the pattern of WM sparing. A major contributing factor to the cardiac remodeling observed in the clinical settings is the extreme inactivity and sedentary lifestyle, which ultimately leads to cardiac unloading. It is well documented in pre-

clinical and clinical studies that cardiac unloading induced by bed rest and transection SCI leads to reductions in the left ventricle internal diameter and reduced cardiac flow indices (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Squair, West et al. 2016). Since the T3-MILD group regained weight supported stepping by one week post injury this ultimately results in sustained cardiac pre-load and positively influences cardiac function. When considering the pattern of WM spared, it is likely that supraspinal control to sympathetic preganglionic neurons is still present. Descending vasomotor control from supraspinal centers such as the rostral ventrolateral medulla (RVLM) travel within the dorsolateral funiculus of the SC and likely remained intact based (Fig.14) (Furlan and Fehlings 2008). Indeed the SCI itself would directly injure preganglionic sympathetic soma located at the T3 segment but the majority of cardiac control would be spared at the T1 and T2 levels (Strack, Sawyer et al. 1988). The spread of the T3-MILD injury was also not sufficient to disrupt a significant amount of sympathetic control from levels rostral or caudal to the injury as it damage did not extend beyond T2 (Fig.14).

We then demonstrate with the follow up experiment that a T2-MOD but not a T3-MOD injury induced persistent cardiac dysfunction. In our follow up experiment the injury severity was increased to further disrupt descending tracts in the dorsolateral funiculus (i.e. T3-MOD) and moved rostral one spinal segment (i.e. T2-MOD) to damage a larger bed of cardiac preganglionic neurons. However, only the T2-MOD injury produced sufficient preganglionic disruption. By six weeks post-SCI the T3-MOD injury systolic function (SV and CO) recovered such that there was no statistical difference from pre-injury levels. The T2-MOD injury was the only injury that elicited evident systolic dysfunction at six weeks post-SCI. Interestingly both MOD injuries resulted in

incredibly similar locomotor deficits as assessed by the BBB (average T3-MOD BBB=11 and average T2-MOD BBB=10). However, the T3-MOD did not induce sustained systolic dysfunction, which maybe due to the level at which the injuries were administered. The majority of cardiac sympathetic preganglionic neurons arise from the T2 and T1 spinal segments (Strack, Sawyer et al. 1988). This suggests to us the critical role of lesion level on chronic manifestations of cardiac function. It is well known that the severity of cardiac abnormalities is lesion level dependent whereby the higher level lesions are associated with the greatest dysfunctions. Since both injuries resulted in similar locomotor function, demonstrated by six week BBB, the initial sympathetic disruption elicited by the T2 coupled with the locomotor deficits is what ultimately lead the presence of chronic systolic dysfunction.

Although T2-MOD SCI induced systolic dysfunction, we did not observe similar cardiac structural remodeling as reported with T3 and T5 transection models (Lujan, Janbaih et al. 2014, West, Crawford et al. 2014). This is possibly due to the duration of the experiments conducted but also maybe a result of differences in the injury models (transection vs. contusion). From an injury induced dysfunction stand point, the T3 transection utilized by West et al. disrupts the majority of descending cardiac sympathetic control which contributes greatly to the observed resting systolic dysfunction. Whereas, the T5 transection utilized by Lujan et al. spares the majority of cardiac sympathetic innervation and would explain the maintained cardiac function observed. From an activity point of view, both T3 and T5 transection *completely* paralyze the lower limbs, which lead way to cardiac unloading. Chronic unloading (i.e. reduced venous return and reduced end-diastolic wall stress) of the myocardium leads to structural remodeling

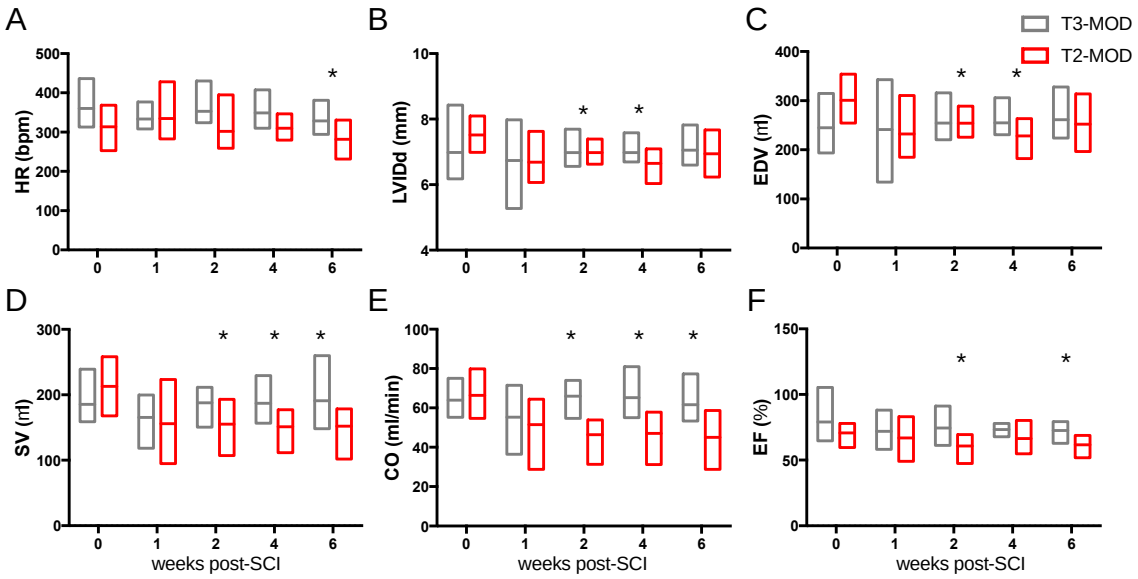
(Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Perhonen, Zuckerman et al. 2001). However, it has been shown that it takes cardiac atrophy six weeks to occur in individuals restricted to best rest although reductions in EDV occur after two weeks (Perhonen, Franco et al. 2001). This suggests that an extreme amount of inactivity is needed to induce cardiac structural remodeling to the degree reported in the chronic SCI population. Transection models mimic that extreme amount of inactivity but are not anatomically representative of the majority of SCI. All of the experiments conducted only lasted six weeks post injury and although BBB locomotor scores did plateau by three weeks post-SCI we did not observe a clear plateau in either the EDV or SV six weeks following T2-MOD injury. Furthermore, EDV is an indicator of the preload placed on the heart and provides a benchmark for the amount of blood being redistributed from the periphery to the myocardium. Since there was no statistical difference in EDV, the maintenance of preload may provide sufficient stimulus to maintain cardiac health and would be maintaining chamber dimensions.

Since T2-MOD SCI produced sustained diminished cardiac function at six weeks post-SCI, we expected there to be altered function in the peripheral vasculature as well. Contrary to this assumption there was no significant SCI induced change in the femoral artery responses to dilators. However, there was a leftward shift such that half the maximal effective concentration (EC_{50}) of ACh response that approached significance ($p=.081$). A possible explanation for leftward shift in femoral artery dose response to ACh is increased muscarinic-three receptor responsiveness. It has been suggested that the potentiated responses to vasoconstrictors is due to the lack of neuronal uptake of adrenergic agonists (Brock, Yeoh et al. 2006, Laird, Finch et al. 2008) rather than just a

change in receptor sensitivity but this could not be the case since vessel responses were produced in a isolated preparation. Furthermore, hypersensitivity to adrenergic agonists (i.e. phenylephrine) in vessels caudal to the level of the lesion has been reported (Yeoh, McLachlan et al. 2004, Brock, Yeoh et al. 2006, Rummery, Tripovic et al. 2010) and it is therefore not unreasonable to suggest that responses to ACh would also be exaggerated. However, the response to SNP, a NO donor, was similar between CON and SCI. This suggests to us that the sensitivity of smooth muscle cells to NO itself is not altered but that changes are occurring within the endothelial cell layer. Furthermore, both pre-clinical and clinical studies have reported reductions in arterial diameter that manifest rapidly after immobilization. Reports of reductions specifically within the femoral artery show the diameter decreases substantially following extreme instances of inactivity such as lower limb immobilization via leg casting and as early as three weeks post SCI (Sugawara, Hayashi et al. 2004, de Groot, Bleeker et al. 2006). This decrease in diameter could potentially increase the wall shear stress (i.e. tangential force) placed on the arterial walls and endothelial cells as blood passes through the lumen ultimately damaging the endothelial cells themselves.

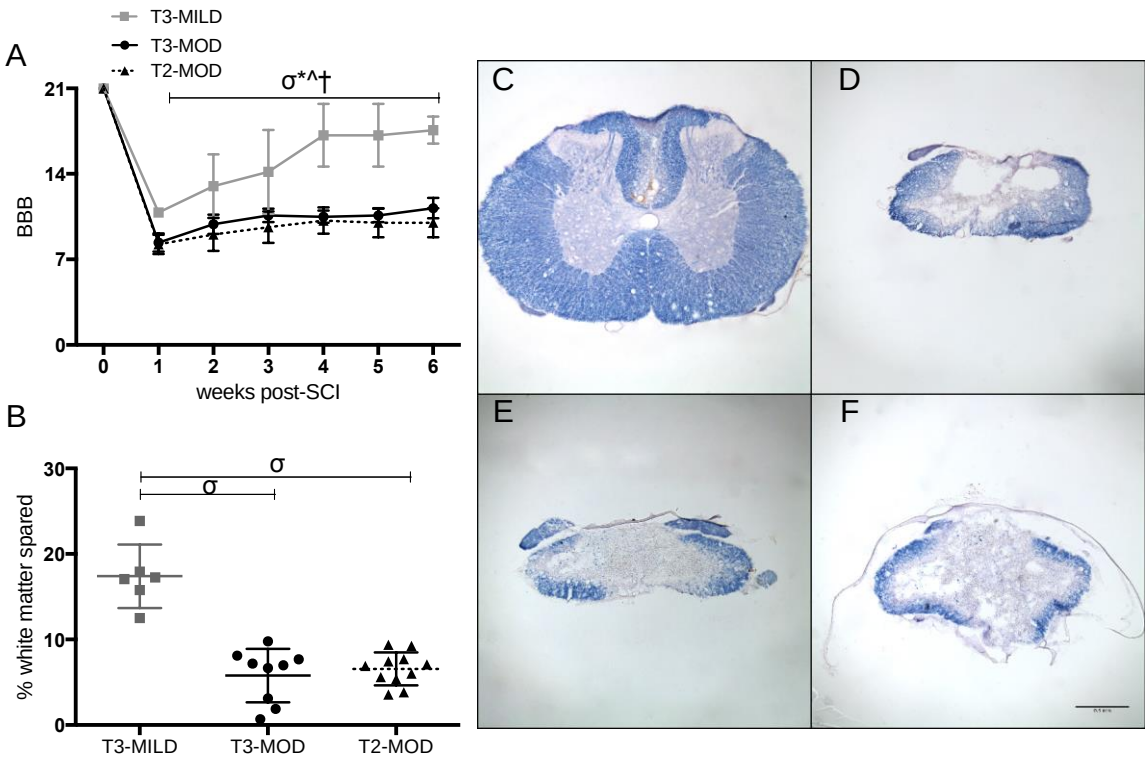
In summary, this data highlights the lack of cardiac decline induced by mild, lower level injuries and the differences in cardiac dysfunction induced by transection vs. contusion injury models. Using a T2-MOD injury, we were able to induce sufficient cardiac decline that persisted six weeks post-injury. Although this specific injury severity resulted in diminished flow derived cardiac metrics, when administered at the T3 level it was insufficient to produce cardiac decline confirming that both the level *and* the severity of the injury greatly influence chronic cardiac function. Whist cardiac decline persisted at

six weeks post T2-MOD SCI, there were no significant changes in vessel responses to vasodilators in large conduit arteries below the level of lesion. Collectively these results represent a significant step forward in our understanding of the limitations of mild contusion injuries and demonstrate the need for future vascular studies to be focused on smaller arteries and arterioles.



<u>Figure 10</u>. Cardiac function is reduced in the T2-MOD but not the T3-MOD group at six weeks post-SCI.

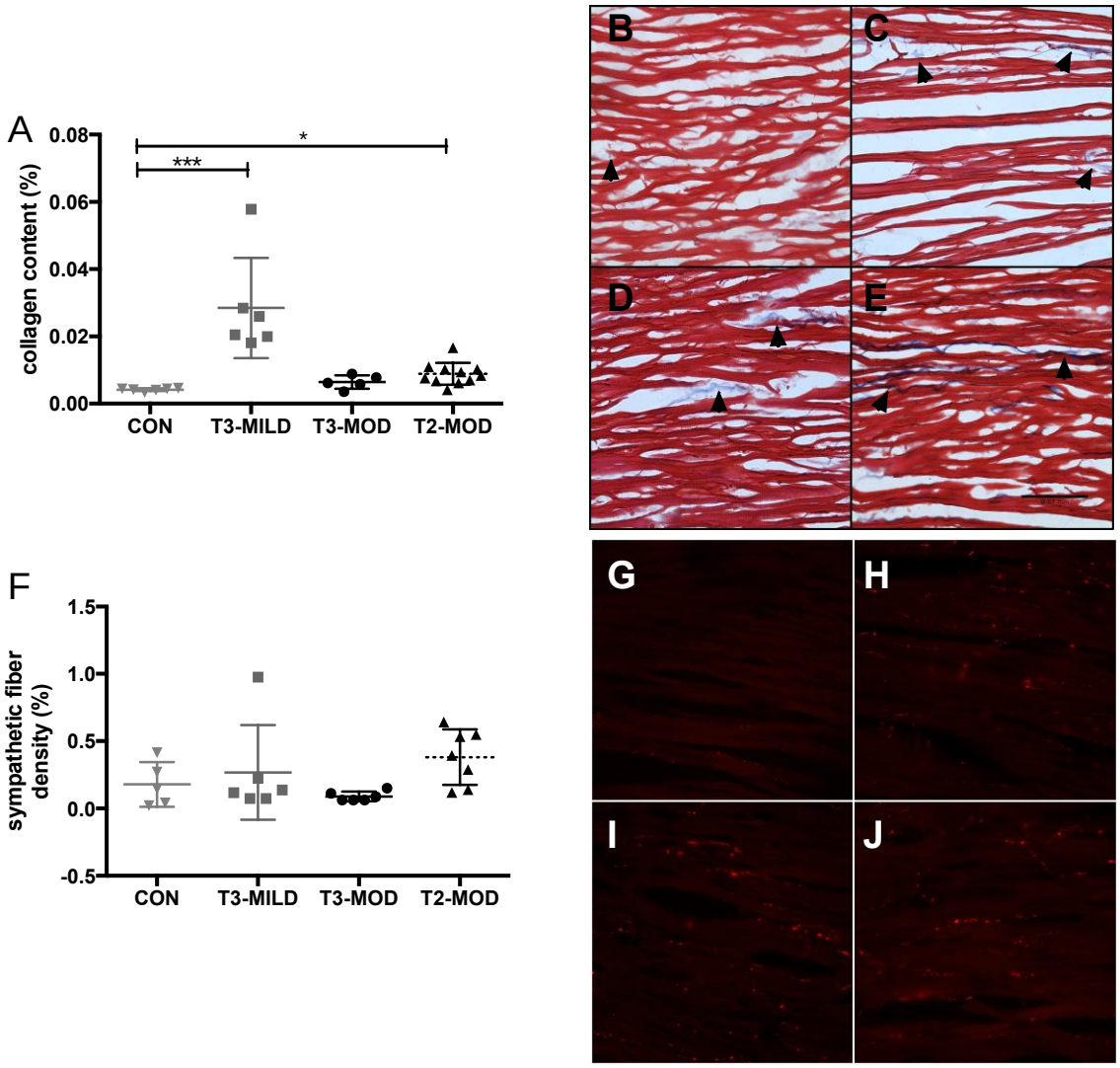
A. Left ventricular internal diameter during diastole (LVIDd). B. Ejection fraction (EF). C. Heart rate (HR). D. End diastolic volume (EDV). E. Stroke Volume (SV). F. Cardiac output (CO). Data are represented as group data with means (bars). *p<.05 T2-MOD vs. pre-SCI.



<u>Figure 11</u>. Between-group comparisons of the weekly locomotor assessments and white matter spared from T3-MILD, T3-MOD and T2-MOD groups.

Weekly locomotor assessments revealed that the T2-MILD group had greater impairments in over-ground stepping compared to both moderate groups during the first weeks post-SCI; although, there was no between group difference at week six (panel A). Moderate injuries resulted in significantly more damage to the white matter (WM) as compared to the mild injury (panel B). C-F. Representative images of WM sparing from one animal in each SCI group. A T2 uninjured CON spinal segment (panel C).The T3-MILD injury resulted in 17.41± 3.7 percent spared white matter (panel D) while the T3 and T2-MOD resulted in 5.79 ± 3.1 and 6.5 ± 1.92 percent WM spared respectively (panel E&F respectively). Data are represented as means ± SD. * P<.05 T3-MILD vs.

pre-SCI; ^ P<.05 T3-MOD vs. pre-SCI; † P<.05 T3-MILD vs. pre-SCI; σ P<.05 T3-MILD vs. MOD injuries.



<u>Figure 12</u>. Histological and immunohistochemical comparisons of the spinal cord and heart between T3-MILD, T3-MOD and T2-MOD.

Analyses demonstrated that collagen was significantly increased in several SCI group (panel A-E) but that sympathetic innervation was not increased compared to CON (panel F-J) Collagen deposition in the T3-MILD injury was significantly increased compared to all other groups (panel A&D). *P<.001 vs T3-MILD; ***P<.0001 vs. T3-MILD.

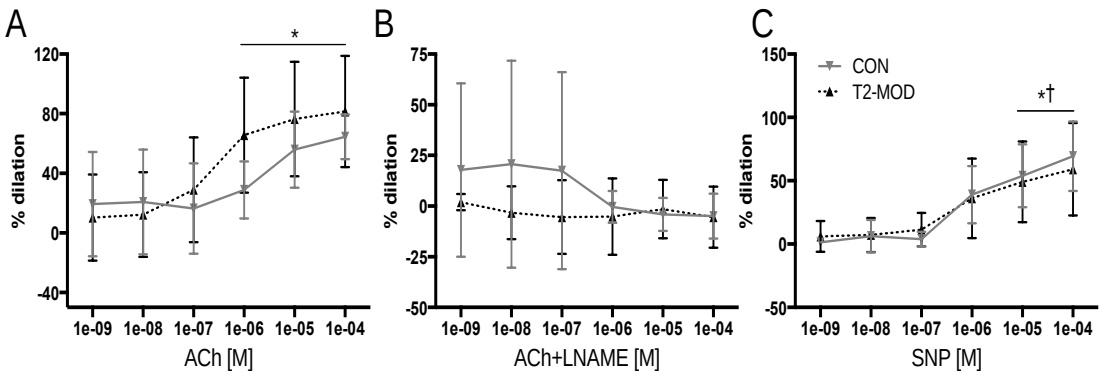
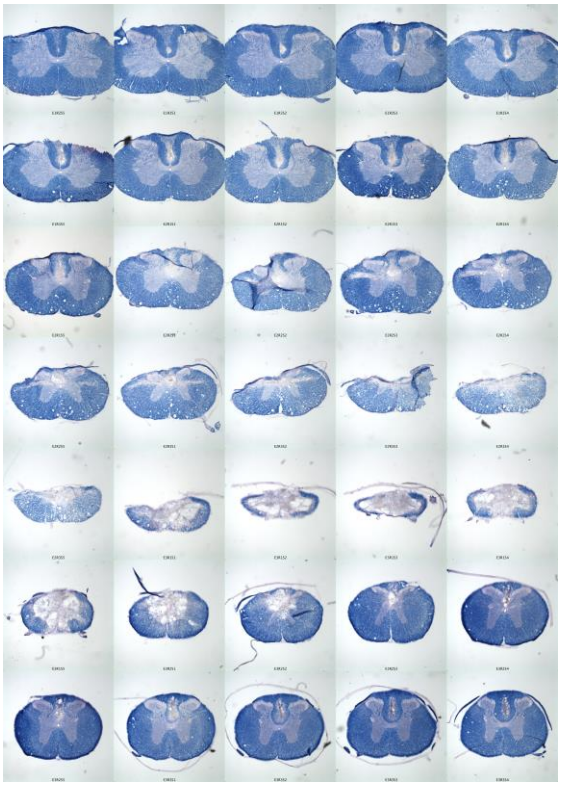


Figure 13. Vascular responses in isolated femoral arteries to endothelial dependent and independent vasodilators are not altered six weeks post T2-MOD SCI.

A. Vessel response curves of CON and T2-MOD SCI to Acetylcholine (ACh). B. Vessel response curves of CON and T2-MOD SCI to ACh and L-NG-Nitroarginine methyl ester (ACh+L-NAME). C. Wessel response curves of CON and T2-MOD SCI to Sodium nitroprusside (SNP). Data are represented as mean ±SD. * P<.05 vs. SCI 1e-09; † P<.05 vs. CON 1e-09.



<u>Figure 14.</u> The spared white mater pattern from T2-MOD injury group.

Table 1. Terminal anatomical and echocardiographical data from T3-MILD, T3-MOD and T2-MOD injury groups.

	T3-MILD	T3-MOD	T2-MOD
Anatomical data			
Body weight (g)	291 ± 17	266 ± 6	259 ± 14
Heart weight (g)	1.04 ± 0.09	1.03 ± 0.09	0.97 ± 0.18
Heart weight/femur	1.17 ± 0.15	0.28 ± 0.02	0.27 ± 0.02
Collagen Content	0.028 ± 0.015	0.01 ± 0.002	0.0100 ± 0.001
Echocardiogrpahic data			
Dimensions			
LVIDd (mm)	5.5 ± 0.7	4.3 ± 0.4	6.2 ± 0.7
LVIDs (mm)	2.8 ± 0.4	2.4 ± 0.2	4.0 ± 0.5
EDV (µl)	170.3 ± 37.5	159.0 ± 23.8	226.9 ± 39.6
ESV (µl)	36.1 ± 12.7	42.8 ± 5.8	144.4 ± 36.0
Systolic function			
SV (µl)	134.2 ± 32.9	116.2 ± 25.5	144.4 ± 36.0
EF (%)	72.0 ± 10.8	44.4 ± 5.8	56.6 ± 8.2
CO (ml/min)	49.3 ± 13.7	37.5 ± 4.5	40.5 ± 9.6

LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; CO , cardiac output; E, peak transmitral filling velocity during early diastole; T3-MILD thoracic level three 12.5g-cm SCI; T3-MOD thoracic level three 25g-cm SCI; T2-MOD thoracic level two 25g-cm SCI Data are displayed as mean ±SD

Table 2. EC50 and maximal dilation to Ach and SNP in femoral arteries of T2 25g/cm SCI and CON rats.

	CON		T2, 6 week SCI	
	EC50	maximal dilation %	EC50	maximal dilation %
SNP	1.73 ± $4.05e^{-05}$	69.4 ± 2.48	5.01 ± $5.27e^{-07}$	59.1 ± 3.6.5
ACh	3.34 ± $5.16e^{-06}$	64.6 ± 1.50	3.21 ± $4.71e^{-07}$	81.5 ± 37.4
ACh+L-NAME	1.00 ± $0.00e^{-9}$	-2.44 ± 5.45	1.00 ± $0.00e^{-9}$	-5.50 ± 15.1

SNP, Sodium nitroprusside; ACh, Acetylcholine; L-NAME ,L-N^G-Nitroarginine methyl ester. Data are represented as means ± SEM.

CHAPTER IV

CHALLENGING CARDIAC FUNCTION POST-SPINAL CORD INJURY WITH DOBUTAMINE

Introduction

Cardiovascular (CV) dysfunction is the leading cause of morbidity and mortality in the chronic SCI population (Garshick, Kelley et al. 2005). Following high thoracic and cervical injuries descending sympathetic control of the vasculature and heart is disrupted leading to a dysregulation of blood pressure and heart rate (HR) and ultimately to cardiovascular decline. Cardiac dysfunction is further compromised by a reduction in demand due to immediate immobility and prolonged inactivity. In individuals with chronic tetraplegia, for example, cardiac atrophy (Kessler, Pina et al. 1986) is prevalent but can be partially reversed with appropriate training (Nash, Bilsker et al. 1991).

Despite the prevalence of cardiac dysfunction in spinal cord injured individuals (Kessler, Pina et al. 1986, Hopman, Oeseburg et al. 1992), little is known about cardiac systolic function independent of the disrupted spinal circuitry. West *et al.* demonstrated that animals with a complete spinal transection at T3 have a blunted ability to develop LV pressure during periods of increased filling (West, Crawford et al. 2014) and Lujan *et al.* demonstrated that following a T5 transection there is increased sympathetic support of

HR and rates of contraction (Lujan, Janbaih et al. 2012). It is important to note that while the transection model disrupts all descending and ascending circuitry this model does not reflect the majority of clinical injuries which are anatomically incomplete even when deemed functionally complete (according to the National Spinal Cord Injury Statistical Center).

Dobutamine stress echocardiography (DSE) is a clinical technique used to investigate how the heart responds to drug-induced increases in sympathetic activation (Krahwinkel, Ketteler et al. 1997). Since Dobutamine is a sympathomimetic drug that primarily targets β-1 receptors, it stimulates chronotropy and inotropy (Ruffolo 1987). DSE is classically used as a means to investigate cardiac regional wall motion for detection of coronary artery blockage, ischemia, and myocardial viability (Pellikka, Roger et al. 1995, Lualdi and Douglas 1997, Wu, Ireland et al. 2004, Leite, Oliveira-Pinto et al. 2015). This clinical technique has recently been applied to the rodent (Plante, Lachance et al. 2005) and is being implemented in experimental models to evaluate cardiac dysfunction in a variety of diseases/pathologies (Schneider, Jaquet et al. 2010, Leite, Oliveira-Pinto et al. 2015). Moreover, the rodent responses to DOB infusion strongly resemble those in humans (Plante, Lachance et al. 2005), most likely because the sympatho-excitatory pathways that innervate the heart are similar across rodents and humans making DSE a highly translatable technique.

The primary objective of this study was to investigate cardiac function and functional reserve following high-thoracic SCI by increasing sympathetic activation with a sympathetic agonist (Dobutamine).

Methods

Experimental design

Experiments were conducted on 14 female Sprague-Dawley (SD) rats (age = 15 wks, weight 250-260g). Group size was determined from power analysis calculations. Based on previous work we would expect a group difference in SV (SD=27.62-44.46), the power to detect a true significant difference equals 80.4-85% with a sample size of approximately 6-8. All procedures were approved by the University of Louisville Animal Care and Use Committee. Animals were randomly assigned to one of two groups: uninjured control (CON n=6) or T2 25 g-cm SCI (SCI n=8). Cardiac function was assessed prior to SCI and at 1, 2 and 6 weeks post-SCI. Dobutamine stress echocardiography (DSE) was conducted pre-SCI and at post-SCI weeks 1 and 6. Hindlimb function during overground locomotion was assessed weekly using the BBB Open Field Locomotor Scale as described previously (Basso, Beattie et al. 1995, Magnuson, Smith et al. 2009).

Spinal Cord Injury Surgery

For SCI surgery, animals were anesthetized with a ketamine/xylazine/acepromazine cocktail (50/0.024/0.005 mg/kg i.p.) and a dorsal mid-line incision was made through the skin and musculature overlying the C8 to T4 spinal segments. A laminectomy was performed at T1 and T2 to expose the underlying T2 spinal cord and moderately-severe injuries (25g-cm) were delivered with a MASCIS Impactor (Rutgers University, NJ). All animals were given Buprenorphine (0.1 mg/kg, SC) twice a day for three days, gentamycin (Gentamicin sulfate 15 mg/kg SC) once a day

for seven days, and 5mL of Lactated Ringers for five days and as needed for hydration. Bladders were expressed manually for five days or until their bladders emptied spontaneously. After recovery, animals were housed socially, two per cage, on the same 12-hour light/dark cycle.

Dobutamine Stress Echocardiography (DSE) Assessments

During DSE assessments, animals were maintained at surgical levels of isofluorane anesthesia (1.5-2%). Core body temperature was maintained at 36-37°C and ventilation was monitored with inductance plethysmography. The tail vein was cannulated with a 25-gauge butterfly needle for drug administration. Images were captured along the parasternal short-axis (SAX) at the midventricular level with a high-resolution ultrasound imaging system (VisualSonics VEVO 2100) and probe (24MHz) secured in a stereotactic stand (VisualSonics). Before drug administration, a pre-DOB image was captured for baseline systolic and diastolic measures. DOB was infused at progressively increasing dosages (5, 10 and 20µg/kg/min; rates of: 2.10, 4.25 and 8.55 ml/hr) for four minutes each, using an automated perfusion pump (KD Scientific, Holliston, MA). Four minutes of continuous drug infusion have previously been shown to elicit a maximal response at each dose (Plante, Lachance et al. 2005). M-mode images were captured at the end of each four-minute administration. Anesthesia was then discontinued and the animals were monitored until fully recovered. Results for 10 cardiac cycles during expiration along the SAX were averaged for between group and dose response comparisons (West, Crawford et al. 2014).

Histology

For histological analyses, animals were perfused with phosphate buffered saline via the ascending aorta to preserve cardiac tissue. The heart was then cleaned of excess fat and vessels and was weighed before being placed in 4% PFA. After 24 hours in fixative, hearts were cryoprotected in 30% sucrose with sodium azide for two days and then blocked in cryoprotective media. The entire spinal column was also extracted and placed in 4% PFA for three days. The SC was then removed from the column and cryoprotected in 30% sucrose for at least 48 hours.

Hearts were sectioned at 10µm and processed for collagen deposition with conventional Masson's Trichrome stain. Images were captured at 20X magnification from the left ventricle free wall from five different sections at least 70µm apart using consistent camera settings. Collagen deposition was quantified as a percent of the total area ($40\mu m^2$) of the image. Specifically, images were set to a threshold to identify collagen-positive tissue and the area of collagen-positive tissue was divided by the total area of the image (in pixels). The percent of collagen was averaged across the five images captured per animals to obtain one data point per animal.

Spinal Cords (SC) were sectioned through the epicenter at 30µm. Sections were allowed to air dry for 30min before storing at 4°C overnight. The following day, SC sections were warmed for 20min and processed for spared white matter using Eriochrome Cyanin (EC) stain as described previously (Smith, Burke et al. 2006).

Statistical Analysis

Repeated measures analyses of variance (RM ANOVAs) were preformed to determine significant main effects and significant interactions between the main effects. For all analyses, parametric ANOVA assumptions were tested (normality and Mauchly's sphericity test). The Greenhouse-Geisser correction was used to adjust degrees of freedom and correct the p value when the variance test was significant revealing unequal variance. Following significant main effects, Tukey HSD *post hoc* t-tests for multiple comparisons were performed on the relevant comparisons of interest to decrease the occurrence of type 1 errors.

Between group comparisons (CON vs. SCI) for echocardiography assessments without Dobutamine were analyzed with RM ANOVA with one factor for time post-SCI (repeated) and one factor for group (independent). Dobutamine stress echocardiography responses were analyzed using RM ANOVA with repeated factors for time post-SCI (i.e. pre-SCI, 1 and 6 weeks post-SCI) and dose.

Anatomical parameters were analyzed with Independent t-tests between means with equal or unequal variance, as appropriate. Statistical analyses were performed with SPSS (v22). Data are displayed as means ± standard deviation (SD). Significance was set at $P \leq .05$.

Results

Our between group analysis revealed that stroke volume (SV), end diastolic volume (EDV) and cardiac output (CO) were all reduced at one week post-SCI compared to age matched CON (Fig.15*A,C&E*; all $P < .05$). At two weeks post-SCI ejection fraction (EF) was also reduced compared to CON (Fig.15*D*; $P < .05$). By six weeks post-SCI,

cardiac flow indices (SV, CO & EF) and HR were all diminished compared to CON levels (Fig.15*A-D*; all P<.05). End diastolic volume was reduced compared to CON (Fig.1*E*; P<.05) although end systolic volume was no different. Relative wall thickness (RWT) and posterior wall thickness (PWT) remained unchanged after SCI.

Our within group analysis revealed that with DOB administration pre-SCI there was a dose-dependent increase in HR and CO (Fig.16*B,C*; P<.05 0 vs. 5,10 & 20µg) but not in SV (Fig.16*A*; P=1.0). End systolic and diastolic volumes (ESV, EDV) decreased with increasing concentrations of DOB (Fig.16*E,F*; P<.01 0 vs. 20µg), which resulted in an increase in the overall EF (Fig.16*D*; P<.05 0 vs. 5,10 & 20µg). Responses to DOB in the CON group were synonymous to those at pre-SCI and did not differ over time.

At one and six weeks post-SCI, DOB administration elicited a dose-dependent increase in HR and CO (Fig.16*B*, HR P< .01 0 vs. 10 and 20µg; *C*, CO P<.01 0 vs. 10 & 20µg) as seen pre-SCI. Ejection fraction also increased with DOB at one and six weeks post-SCI (Fig 16*D*; P<.05 0 vs. 5µg; P<.01 0 vs. 10 and 20 µg). End systolic volume decreased with increasing DOB concentrations (Fig.16*F*; all P<.01 0 vs. 5,10 & 20µg) and while there appeared to be a decrease in EDV, the changes were not statistically significant. Unlike pre-SCI responses, DOB induced a substantial increase in SV at one and six weeks post-SCI (Fig.16*A*; P< .05 0 vs. 5; P<.01 0 vs. 10 & 20 µg). In fact, SV and CO were no longer different from pre-SCI baseline (0µg) with 5µg/kg dose of DOB (P=.058 and .056 respectively) We also found an interaction effect between group and dose whereby the magnitude of change in CO from baseline (0ug) to 5µg was much larger 6 weeks post-SCI group pre-SCI (Fig.16*A*&*C*; P<.005 interaction effect between time and dose).

Histological assessments demonstrated an increase in collagen deposition following SCI compared to uninjured, age-matched controls (Fig17. *E&F* P <.005) The heart wet mass was also diminished compared to controls (Table 3. P<.05). The 25g-cm injury resulted in 6.25 ± 1.9% spared white matter at the epicenter (Fig.17*B*).

By 6 weeks post-SCI, hindlimb function had recovered sufficiently to allow occasional weight-supported stepping without forelimb-hindlimb coordination (Fig.17; BBB = 9.9 ± 1.2;) (Basso, Beattie et al. 1995). While we did not stratify our animal groups based on locomotor recovery, we could find no significant correlations between the BBB scores and indices of CO (Fig.16R^2=0.02575, P=0.7042).

Discussion

The main finding of this study was that cardiac systolic responses to beta-adrenergic stimulation (examined using DSE) are exaggerated at six weeks following a contusive SCI and that DOB administration elicited an increase in SV at six weeks post-SCI that did not occur pre-SCI. In addition, we extend observations of attenuated systolic function and cardiac fibrosis made in the complete T3 transection SCI model in adult male Wistar rats (West, Crawford et al. 2014) to adult female Sprague-Dawley rats with incomplete contusion injuries that allowed the recovery of occasional weight-supported hindlimb stepping (BBB = 9.9). We utilized T2, 25g-cm contusions that spared greater than 6% white matter cross-sectional area at the epicenter. Interestingly, we also demonstrate with a 5µg/kg dose of DOB SV and CO are returned to pre-SCI levels.

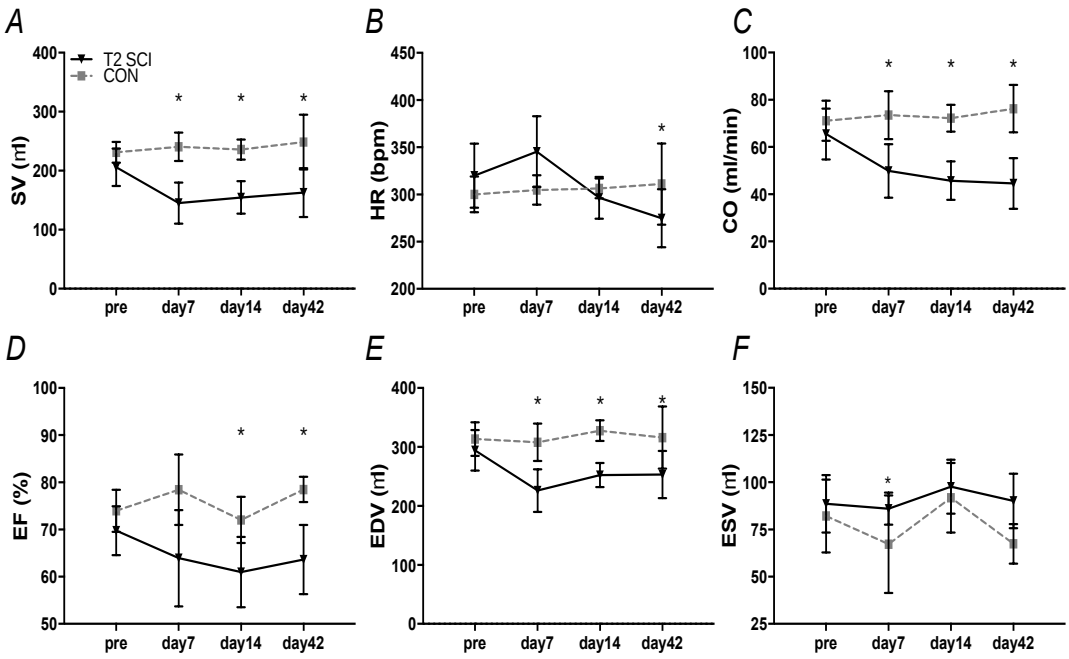
Rodent models with T3 and T2 injures have previously shown clinically relevant cardiovascular impairments such as reduced systolic function and altered hemodynamics

(West, Crawford et al. 2014, Squair, West et al. 2016). Here, we found that reduced systolic function (CO, SV and EF) was present at 1 week post-SCI and persisted at this low level at 6 weeks post-SCI. Such a rapid and sustained response with the T2 25g-cm injury is in agreement with these previously defined models and mimics the clinical scenario (Kessler, Pina et al. 1986, Eysmann, Douglas et al. 1995). Reduced left ventricle internal diameter (LVID; Table 3.) is most likely due to the diminished hind-limb function and subsequent cardiac unloading. This is in concert with human studies that have shown prolonged episodes of bed rest (i.e. cardiac unloading) result in decreased LVID, EDV and SV (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Perhonen, Zuckerman et al. 2001). Furthermore, we believe the sustained reduction in HR six weeks post-SCI (Fig.15) is a direct indication of altered autonomic balance in favor of overriding vagal tone (West, Romer et al. 2013). This is in agreement with the clinical population where individuals with tetraplegia present with lower resting HRs compared to able bodied and paraplegic individuals (Zhu, Galea et al. 2013, Currie, West et al. 2016). The administration of DOB, which bypasses the disrupted afferent circuitry, corrected these deficits.

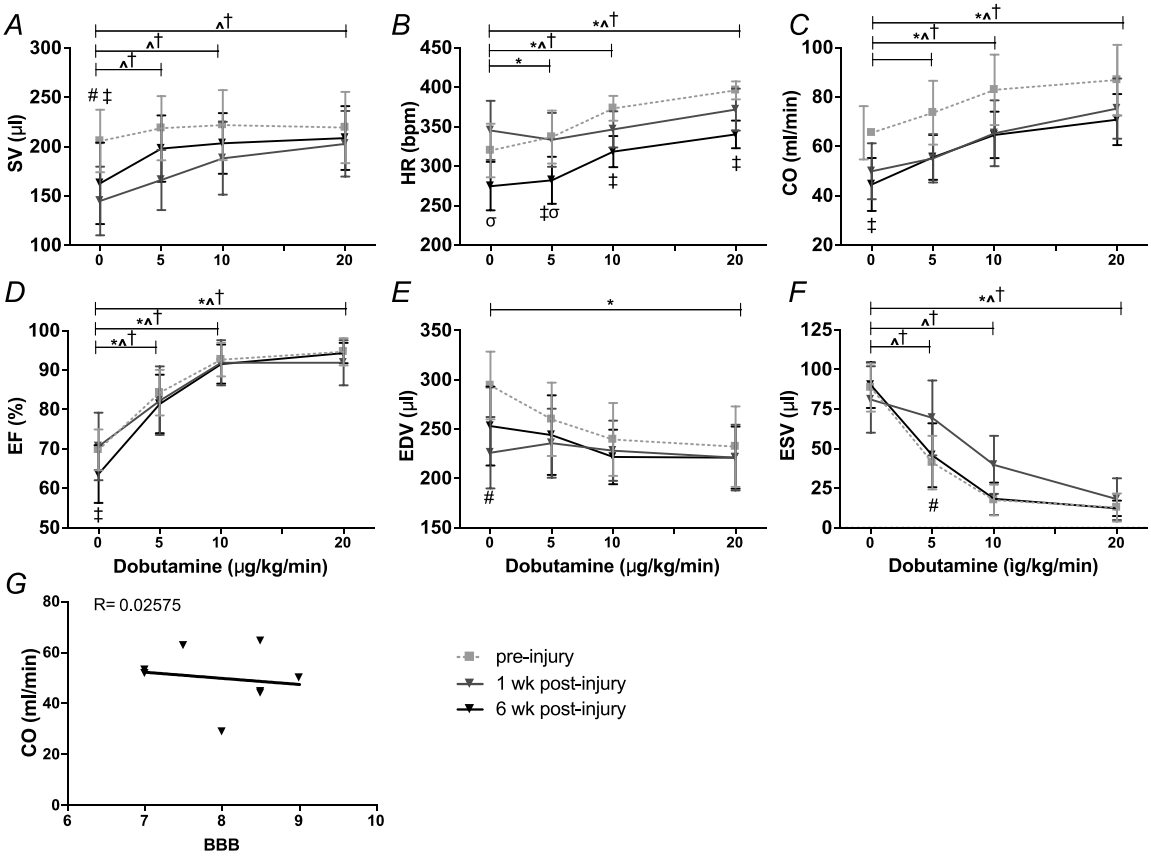
In uninjured male rats, DOB elicits an increase in CO (lead by HR) and EF along with decreases in EDV and ESV (Plante, Lachance et al. 2005) which mirrors DOB responses in our uninjured female rats and in the able bodied clinical population (Ruffolo 1987, Pierard, Berthe et al. 1989). In the current study, we demonstrated that uninjured females (pre-SCI) respond in a similar pattern (Fig.16&3*A*-*F*). We then demonstrated that female rats with contusive spinal cord injuries respond to DOB administration with an increase in CO due to increases in both HR *and* SV (Fig.16 *A, B*), suggesting that reduced

SV at rest is likely a direct consequence of reduced descending sympathetic control. Arguably one of the most important findings in the present study was that animals with chronic SCI (i.e. 6 week data) exhibited an exaggerated response to low doses of DOB (5µg/kg) compared to the pre-SCI and one-week data. While the mechanism underlying this increased responsiveness is not clear, it is possible that there are chronic changes in the number/sensitivity of cardiac beta-receptors that are not present at one week post-SCI but manifest by six weeks. Although no studies have directly investigated this hypothesis in the heart, similar findings have been noted in the alpha-adrenergic system where enhanced reactivity to norepinephrine and other alpha-mimetics were observed post-SCI (Mathias, Frankel et al. 1976, Krum, Louis et al. 1992, Yeoh, McLachlan et al. 2004). An important consideration in the present study is that our DSE protocol failed to achieve a plateau in HR, SV or CO (maximum work) most likely due to an inability of the 20µg/kg/min dosage to reach the upper limits of the Starling curve. Thus, we were unable to observe the full impact of the consequences of the measured myocardial fibrosis (i.e., increased collagen deposition). We had expected, based on previous work (Conrad, Brooks et al. 1995), that cardiac fibrosis would increase the stiffness of the left ventricle and subsequently reduce contractile function/responsiveness. We also cannot speak to the comprehensive hemodynamic responses to DOB, because we did not assess blood pressure responses during drug administration. However, it is well known that DOB elicits an increase in systolic pressure which quickly stabilizes (Plante, Lachance et al. 2005) and does not effect overall peripheral resistance (Segreti, Marsh et al. 2008, Badea, Hedlund et al. 2011).

This is the first study, to our knowledge, to demonstrate persistent impairments in cardiac function following a contusive spinal cord injury (T2 25g-cm) that are similar to those seen after a T3 complete transection. We observed increases in cardiac fibrosis and attenuated systolic function six weeks post-SCI, confirming previous findings (West, Crawford et al. 2014). Moreover, using DSE we observed an increased responsiveness of the heart to low doses of a beta-agonist post-SCI as compared to pre-SCI. Specifically, we found that a low-dose administration of DOB resulted in an increase (and normalization) of SV, confirming for the first time that impaired descending control of the heart is directly contributing to reduced resting SV after SCI. Due to the similar neuroanatomical innervation of the heart between human and rodent (Strack, Sawyer et al. 1988, Dampney 1994), we believe these findings are easily translatable and applicable to the clinical scenario

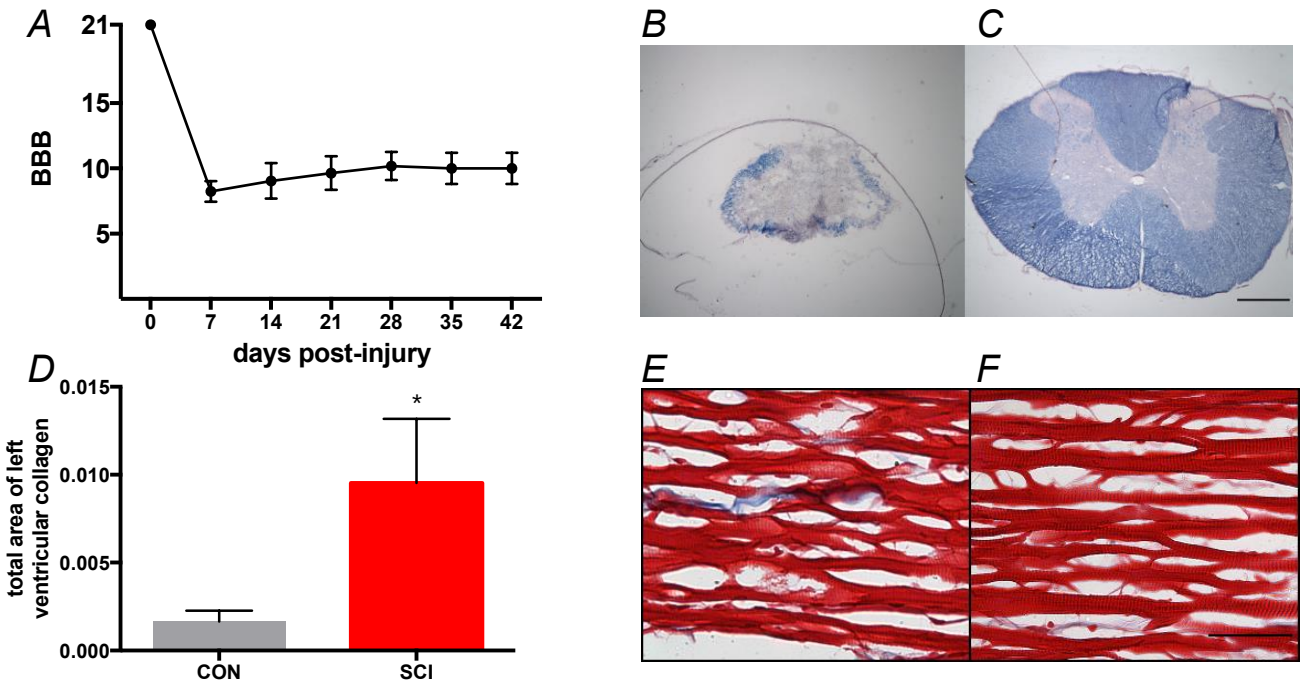


<u>Figure 15</u>. Cardiac function of the T2 25g-cm spinal cord injury (SCI) female SD rats and age matched uninjured controls (CON) followed for six weeks post-SCI. A, stroke volume (SV). B, heart rate (HR). C, cardiac output (CO). D, ejection fraction (EF). E, end diastolic volume (EDV). F, end systolic volume (ESV). Data are represented as means ± standard deviations. * P≤.05 SCI vs. CON.



<u>Figure 16</u>. Responses to increasing doses of Dobutamine pre-SCI and at one and six weeks post-SCI.

A, stroke volume (SV). B, heart rate (HR). C, cardiac output (CO). D, ejection fraction (EF). E, end diastolic volume (EDV). F, end systolic volume (ESV). G, correlation of locomotor function with CO six weeks post-SCI (Pearson correlation R=0.02575). There was a significant main effect for dose and time in all outcome measures (P<.05). There was a significant interaction effect for time and dose in all outcome measures (P<.05). Data are represented as means ± standard deviations. * P<.05 pre-SCI 0 µg vs. all other doses, ^ P<.05 1 week post-SCI 0 µg vs. all other doses, † P<.05 6 week SCI 0 µg vs. all other doses, # P<.05 pre-SCI vs. 1 week post-SCI, ‡ P<.05 pre-SCI vs. 6 weeks post-SCI, σ P<.05 1 week post-SCI vs. 6 weeks post-SCI.



<u>Figure 17</u>. The BBB scores of the SCI group followed for six weeks after SCI and the histological comparison of SCI groups to the CON group.

A, Locomotor recovery from injury to six-weeks post-SCI. B&C, Representative images of white matter spared at the injury epicenter from and SCI and CON animal, respectively. Scale bar is 500µm. E&F, Representative images of collagen in the left ventricle free wall of CON and SCI animals, respectively. Scale bar is 50µm.

Table 3. Anatomical and echocardiographic data for control and SCI rats at 42 days post-SCI.

	T2 SCI (n=8)	CON (n=6)
Anatomical data		
Body mass (g)	259.13 ± 14.49	272.17 ± 7.86
Heart mass (g)	1.013 ± 0.18†	1.08 ± 0.11
Heart mass/femur	1.22 ± 0.22	1.42 ± 0.27
Collagen Content	0.0095 ± 0.0013††	0.0016 ± 0.0003
Echocardiographic data		
Dimensions		
LVIDd (mm)	6.95 ± 0.49 †††	7.67 ± 0.56
LVIDs (mm)	4.44 ± 0.30 ††	3.92 ± 0.26
EDV (μl)	253.03 ± 40.0 †††	315.9 ± 52.57
ESV (μl)	90.13 ± 14.45 ††	67.42 ± 10.47
Systolic function		
SV (μl)	162.89 ± 41.27 †††	248.48 ± 46.44
EF (%)	63.64 ± 7.34 †	78.49 ± 2.68
CO (ml/min)	44.56 ± 10.73 †	76.25 ± 10.06
Diastolic function		
E (cm s-1)	63.19 ± 8.621†††	81.23 ± 16.82
A (cm s-1)	46.011 ± 9.42	36.58 ± 23.7
E/A	1.4 ± 0.17†	2.22 ± 0.71

LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; CȮ, cardiac output; E, peak transmitral filling velocity during early diastole; A, peak transmitral filling velocity during late diastole; CON, uninjured control; SCI, spinal cord injury; Data are displayed as mean ±SD ††† P<.001 vs. CON; †† P<.01 vs. CON; † P<.05 vs. CON

CHAPTER V

A COMPARISON OF PASSIVE HIND-LIMB CYCLING AND ACTIVE UPPER-LIMB EXERCISE PROVIDES NEW INSIGHTS INTO CARDIAC DYSFUNCTION FOLLOWING SPINAL CORD INJURY

Introduction

It is well documented that high thoracic and cervical spinal cord injury (SCI) leads to systolic cardiac dysfunction (Kessler, Pina et al. 1986, West, Campbell et al. 2012), altered hemodynamics (Kessler, Pina et al. 1986, West, Mills et al. 2012, Wecht, Zhu et al. 2013, West, Crawford et al. 2014, Squair, West et al. 2016), and cardiac atrophy (Kessler, Pina et al. 1986, Eysmann, Douglas et al. 1995, de Groot, Bleeker et al. 2006). Cardiac dysfunction is thought to arise from a combination of a decentralized sympathetic nervous system (Furlan, Fehlings et al. 2003, West, Crawford et al. 2014) and significant cardiac unloading due to reduced vascular tone (Wecht, de Meersman et al. 2000, Krassioukov 2009), reduced circulating blood volume (Houtman, Oeseburg et al. 2000) and a loss of muscle (respiratory and skeletal) pumps (Faghri and Yount 2002). Upper- and lower-limb exercise has been applied in the chronic and acute setting with a view to offsetting the decline in heart function. However, the cardiac outcome measures of choice in prior studies have traditionally focused on global indices of systolic and diastolic function obtained from echocardiography. Although these measures can be used to track temporal changes in cardiac function, they are load dependent and provide little

insight into intrinsic cardiac and left ventricle (LV) contractile function. Hence, it is currently unknown in SCI whether exercise enhances left-ventricular contractile function, or whether exercise improves cardiac outcomes via improved/altered loading conditions.

There is general agreement in the scientific literature that upper-limb exercise alone does little to mitigate cardiac decline (Davis, Shephard et al. 1987, Nash and Jacobs 1998, Gates, Campbell et al. 2002, West, Currie et al. 2015). However, there is a growing interest in lower limb exercise since it has been demonstrated that SCI-induced systolic dysfunction can be prevented with passive hind-limb cycling (PHLC) in rodents (West, Crawford et al. 2014) and following electrically stimulated cycling in humans (Nash, Bilsker et al. 1991). To date, there has been no direct comparison between PHLC and active upper-limb exercise. Swim training (SWIM) has been widely used in pre-clinical studies and is well characterized as both a form of exercise-associated rehabilitation and as a locomotor assessment (Smith, Burke et al. 2006, Smith, Shum-Siu et al. 2006, Magnuson, Smith et al. 2009, Radovits, Olah et al. 2013). Uninjured rats swim bipedally via alterations of the hind-limbs and utilize forelimbs only for steering and trunk stabilization. However, following SCI, injured rats rely solely on their forelimbs for propulsion (Magnuson, Smith et al. 2009) due to hind-limb paralysis. Therefore, using SWIM and PHLC interventions provides an ideal paradigm to investigate the potential cardio-protective effects of active upper- and passive lower-limb exercise in the setting of SCI.

In the current study, we combine *in vivo* pressure-volume (PV) analysis of the LV with *in vivo* echocardiography to gain a comprehensive understanding of the cardiac changes elicited by PHLC and SWIM training following severe contusion SCI. We also

evaluate the relative contribution of altered sympatho-excitatory control of cardiac function post-SCI throughout the study by examining the cardiac responses to Dobutamine (DOB) administration. Since Dobutamine is a sympathomimetic drug that primarily targets β-1 receptors, it induces sympathetic responses such as chronotropy and inotropy (Ruffolo 1987). This is the first study to directly compare these two clinically used interventions with both load-dependent and -independent measures.

Materials and Methods

Experimental Design

Experiments were conducted on 24 male Wistar rats (250-300g; Harlan Laboratories). Animals were evenly divided into one of four groups; an uninjured control (CON), a thoracic level two (T2) contusion (400KD with 5 second dwell, T2 contusion SCI group with passive hind-limb cycling (PHLC), or a T2 contusion SCI group with swim group (SWIM). All animals underwent standard echocardiography assessments pre-SCI, and one, two and five weeks post-SCI. Dobutamine stress echocardiography was preformed pre-SCI, and one and five weeks post-SCI. At the termination of the experiment (five weeks post-SCI), animals were implanted with a PV conductance catheter to generate *in vivo* recordings of the LV-PV relationships (Fig.18). All procedures were conducted in accordance with the Canadian Council for Animals and ethics were approved by the University of British Columbia.

Spinal Cord Injury Surgery

Rats were prophylactically treated for three days prior to SCI surgery with

enrofloxacin (Baytril; 10 mg kg^{-1}, s.c., AVP). On the day of surgery, rats were anesthetized with isoflurane (5% at induction chamber and maintenance with 2.5%, 1.5-2 L/min Oxygen flow) and administered burpronephrine (0.02 mg kg^{-1}, s.c.), enrofloxacin (10 mg kg^{-1}, s.c.) and warmed lactated ringers (5mL, s.c). The skin overlaying the C7-T3 vertebrae was shaved, cleaned and a dorsal mid-line incision was made through the skin, fascia and muscle layers. The T2 lamina was removed and the spinal cord was exposed. A T2, 400KD contusion with a 5 second dwell was generated by an Infinite Horizon impactor (Precision Systems and Instrumentation, LLC, Fairfax Station, VA). The muscle and skin layers were closed in layers with 4-0 myocryl and 5-0 prolene sutures, respectively. Rats were allowed to recover in a temperature-controlled environment (33°C, Animal Intensive Care Unit, HotSpot for Birds, Los Angeles, CA). For two weeks post-SCI, rats were weighed and monitored daily after which monitoring was performed every other day. Enrofloxacin (10 mg kg^{-1}, s.c.), buprenorphine (0.02 mg kg^{-1}, s.c.) and lactated Ringer's solution were administered for three days following surgery and bladders were manually emptied three to four times daily until spontaneous voiding returned. This surgery and care protocol has been outlined in detail elsewhere (Squair, West et al. 2016).

Exercise Paradigms

Passive hind-limb cycling and SWIM training began 8 days following SCI and lasted for 25 days. The PHLC group cycled 5 days a week for 30 min each day using a custom-made ergometer (Fig.18B). Briefly, rats were horizontally suspended in a leather sling with two holes cut for their hind-limbs. Their hind paws were secured to pedals with

gauze (to prevent skin abrasions) and Parafilm. Rats were cycled at a frequency of 0.5 Hz. The SWIM group swam 6 x 5min sessions 5 days per week, totaling 30min of exercise per day. Animals swam in a custom-made lap pool (Fig.18C; 60”x18”x7”). During each swimming session, animals were placed at one end of the pool and encouraged to swim with aid, by either tail or slight trunk support, to a padded ramp at the other end. Once the animal reached the ramp they were picked up and re-set to the opposite end of the pool to start another swim lap. Pool water was maintained at 32°C for the duration of the swimming sessions and was warmed when needed.

Echocardiography and Dobutamine Stress Echocardiography

Echocardiography was performed with a GE Vivid 7 (GE Medical, Horten, Norway) system and i13L, 5.6-14.1 MHz transducer. Rats were anesthetized with isoflurane and maintained at a surgical depth of anesthetic (induction chamber at 5% with 1.5-2 L/min Oxygen flow followed by 2-1.5% with 1.5-2 L/min Oxygen flow), placed in a supine position and their thorax was shaved. Body temperature was maintained at 37-38°C and HR was monitored with a three-lead electrocardiogram using LabChart 8 Software (ADInstruments, Colorado Springs, CO USA). Standard measures of LV structure and function were obtained along the parasternal short-axis. Pulse-wave Doppler was used to estimate early (E) diastolic filling. Results from five cardiac cycles during expiration were averaged together and used for between group and within group (time-post-SCI) comparisons. Basal echocardiography measurements were normalized to femur length for terminal between group comparisons and are reported in the supplementary Table 4.

For DOB echocardiography assessments, animals were prepped and basal images were captures as described above. Following the acquisition of basal (i.e. 0µg) cardiac measurements, the tail was cleaned and the tail vein was cannulated with a 27-gauge butterfly needle. Dobutamine (Dobutamine, I.V., Hospira) was intravenously infused in a step-wise manner with progressively increasing doses (5, 10, 20 and then 30µg/kg/min) using an automated perfusion pump (Harvard apparatus; Southnatick, MA, USA). The 5µg/kg/min dose was administered for 5 min to reach a peak response and each consecutive dosage was infused for four minutes to obtain a peak response to the drug.(Plante, Lachance et al. 2005) An image was captured along the parasternal short axis during the last 30 seconds of each dose and analyzed off line. Animals were removed from the echocardiography stage and their recovery was monitored in a temperature-controlled environment. Results from five cardiac cycles during expiration were averaged together for each dose response and used for between group and time post-SCI comparisons.

Hemodynamic Measurements: LV-PV loop analyses

Rats were anesthetized with isoflurane (5% at induction with a 1-2 L/min oxygen flow followed by 2.5-2% maintenance), core body temperature was maintained at 37°C, and a three-lead ECG was used to monitor HR through LabChart8 Software. Animals' necks were shaved, cleaned and the right common carotid artery was exposed via a midline neck incision. The pre-tracheal muscles were retracted to provide a clear window to the artery and three silk suture ligatures were placed loosely around it. The most rostral ligature was tied off while the most caudal ligature was elevated to temporarily occlude

the artery. A small incision was made in the artery with a bent tip needle and a 2-Fr microtip pressure-conductance catheter (SPR-869, Millar Instruments, Houston, TX) was inserted and secured in place with the final (middle) suture. The caudal suture was then released and the conductance catheter rested in the right common carotid for five minutes to collect beat-by-beat blood pressure to calculate resting mean arterial pressure (MAP) and systemic vascular resistance (SVR). The catheter was advanced into the LV under pressure guidance and after optimizing the PV signals; 10 minutes of resting data were recorded at 1000Hz (Powerlab, ADInstruments, Colorado Springs, CO USA) and visualized with commercially available software (LabChart8.1 Plus Software, AD instruments). Using specialized PV analysis software (PVAN, Millar Instruments), the end-systolic pressure (ESP), end-diastolic pressure (EDP), the maximal slope of LV systolic pressure increment (dP/dt_{max}) and diastolic pressure decrement (dP/dt_{min}), ejection fraction (EF), and stroke work (SW) were computed and calculated off-line. Stroke volume (SV) and cardiac output (CO) were calculated and corrected according to PVAN software calibrations. To account for the influence of body mass differences, CO and SW derived from PV analysis were also normalized to body mass (cardiac index, CI and SW index, SWI).

After acquiring basal PV measurements, DOB was infused in the same step-wise manner as described above, in progressively increasing concentrations. Pressure and volumetric responses to DOB were analyzed off-line with specialized PV analysis software. Following DOB infusion and data collection, the animals recovered for 10 min before assessing load-independent metrics. The IVC was approached through a mid-line abdominal incision and occluded with a cotton applicator three consecutive times to

generate load-independent measures of LV contractility including the end-systolic pressure volume relationship (Ees), preload recruitable stroke work (PRSW), and the slope of the dP/dt_{max} end-diastolic volume relationship (dP/dt_{max}-EDV). Calibration was then performed in accordance with procedures that have been well described previously.(Pacher, Nagayama et al. 2008) Briefly, the pressure and conductance were calibrated using the internal standard signal calibration. Saline (hypertonic, 50µl) was injected into the already exposed IVC to calculate the conductance volume and correct for cardiac mass volume interference during PV collection. Finally, the conductance signal was calibrated to absolute volumes using a cuvette calibration wells filled with fresh, heparinized, warm blood. All data analysis of PV relationships was conducted in Labchart8 (ADInstruments, Colorado Springs, CO USA). Animals were then overdosed with isoflurane.

Statistical Analyses

Statistical analyses were performed with SPSS (v22, Chicago, IL) and the significance level was set at $P < .05$. All basal cardiac and data (i.e. echocardiography and PV) were analyzed using one-way analysis of variance (ANOVA) or repeated-measures analysis of variance (RM ANOVA) where appropriate. Drug response comparisons were analyzed with RM ANOVA. Load-independent cardiac measures were analyzed using mixed-model regression analyses (STATA v12.1), as recommended in the literature (Burkhoff, Mirsky et al. 2005).

For all analyses, parametric ANOVA assumptions were tested (normality and Mauchly's sphericity test). The Greenhouse-Geisser correction was used to adjust

degrees of freedom and correct the p value when the variance test was significant. All significant main or interaction ANOVA effects were further investigated using Tukey's HSD *post-hoc* testing. Data are represented as mean ± standard deviation unless indicated otherwise.

Results

We first tracked temporal changes in global indices of systolic and diastolic function using echocardiography. We found that by one week post-injury, all SCI groups exhibited a significant reduction in left ventricle internal diameter during diastole (LVIDd) compared to CON (Fig.19A $p<.05$). Volume indices derived from echocardiography, such as CO and SV (Fig.19B&C), were also reduced by one week post-SCI in all SCI groups compared to CON (suppl. Table 4. All $p<.05$). By five weeks post-SCI, both SWIM and SCI groups exhibited reduced volumetric indices compared to CON ($p<.05$) but the PHLC group exhibited CO and SV that was no longer different from CON ($p=1$). We then assessed hemodynamic parameters at study termination (five weeks). We found that severe contusion SCI resulted in significantly lower systolic blood pressure (SBP) and MAP and that neither exercise intervention improved blood pressure (Fig.19D,E). We also found a reduction in HR within all of our groups compared to CON (Fig.19F).

Throughout the study, we assessed the effects of the beta agonist, Dobutamine, on cardiac function. We found that pre-SCI all groups responded similarly to drug administration with an increase in HR and a decrease in EDV (HR $p<.001$; EDV $p<.05$; Fig.20A,B). Stroke volume decreased slightly during drug administration while CO

increased slightly but these changes were not significantly different from basal (0µg) levels (Fig.20C,D). At one week post-SCI, all SCI groups showed a different response profile compared to drug induced changes pre-SCI. Although there was an increase in HR (Fig.20E), there was no decrease in EDV (Fig.20F; SCI P=0.74; PHLC&SWIM P= 1.00) in any SCI group. Although there was a statistically significant SV increase during the 5µg dose in the SCI group, this increase was not maintained during the 30µg administration (Fig.20G). By five weeks post-SCI, HR increased substantially with DOB although the peak HR obtained in the PHLC and SWIM groups was significantly less than the max HR pre-SCI (Fig.20I). Similar to the one week response, there was no significant decrease in EDV in any of the groups (Fig.20J). Interestingly, at five weeks post-SCI, SV increased with DOB in the SWIM and SCI group but not in the PHLC group (Fig.20K). The 5µg dose of DOB elicited an increase in CO in all groups at five weeks, which was maintained at 30µg (Fig.20L).

Finally, at the termination of the study (five weeks post-SCI) we assessed basal hemodynamics, left ventricular function and the pressure generating capacity of the heart with LV-PV catheterization (Fig.21A). We found all groups had lower mean arterial pressure (MAP) compared to CON (Table 4. P<.05). In addition to this, the SCI and SWIM groups had an increased SVR (Table 4. P<.05). We also calculated the low frequency systolic blood pressure (LFSBP) as previously described (Inskip, Ramer et al. 2012) and found that LFSBP was significantly diminished in SCI and that neither exercise intervention influenced its recovery (Fig.22H P<.001). Our LV-PV data confirmed echocardiography findings of reduced volumetric indices in the SCI and SWIM groups and the return of those indices to CON levels with PHLC (Fig.21B).

However, all SCI groups had significantly lower pressure generating capacity (Pes and dP/dt_{max}) compared to CON (Suppl. Table 4.). To separate the effects of loading on LV function, we manipulated cardiac preload via an inferior vena cava occlusion (Fig21.C-F). We found that SCI had a reduced slope of the end systolic pressure volume relationship (end-systolic elastance; Ees), PRSW, and dP/dt-EDV relationships (Fig.21B,C,E&F). Surprisingly, neither PHLC nor SWIM interventions corrected resting pressure generating capacity or load-independent indices (Fig.21D-F). However, when DOB was administered, volumetric (as demonstrated with echocardiography) and pressure indices were restored in all SCI groups (Fig.22). Dobutamine increased end systolic pressure (Pes; Fig.22H) in the SCI and SWIM groups but not the CON and PHLC groups. End diastolic pressure was not affected by DOB administration (all P=1.00). Pressure volume analysis also revealed that SCI and SWIM significantly increases arterial elastance (Ea). Since Ees is also decreasing, both SCI and SWIM exhibited ventriculoarterial (Ea/Ees) decoupling. We also found that PHLC prevented a decrease in Ea, which in turn prevented ventriculoarterial decoupling while SWIM training failed to correct Ea (Suppl. Table 4.).

Discussion

This is the first study to directly compare the influence of two exercise interventions, PHLC and SWIM, on cardiac function following SCI. Left ventricle structure and function was markedly reduced following severe T2 contusive SCI, which manifested as reduced internal diameter, reduced systolic function, attenuated pressure generation, and impaired systolic load independent indices. Passive hind-limb cycling

corrected volumetric cardiac indices while SWIM training did not. However, neither PHLC nor SWIM training improved the pressure generating capacity of the heart or promoted the recovery of load-independent systolic cardiac dysfunction. When DOB was administered at the termination of the study, it improved both pressure and volume (albeit to a lesser extent) indices in all SCI groups.

The first finding of this study was that severe T2 SCI contusion produced a marked reduction in volumetric cardiac indices which were corrected with PHLC but not SWIM training. Reduced LV volumetric indices following SCI is well documented in both pre-clinical and clinical scenarios (Kessler, Pina et al. 1986, Eysmann, Douglas et al. 1995, de Groot, Bleeker et al. 2006, West, Crawford et al. 2014) and is most likely due to chronic unloading of the myocardium. For instance, it has been demonstrated in able-bodied studies that extended periods of bed rest (i.e. cardiac unloading) result in a reduction of LV chamber size (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001). Furthermore, SCI at this level directly disrupts sympathetic outflow to the vascular beds responsible for blood volume redistribution (Strack, Sawyer et al. 1988) and injury-induced paralysis hinders the venous muscle pump from the lower extremities (Raymond, Davis et al. 1999). As such, we expected that the PHLC intervention would reverse the SCI-induced reduction in LV chamber size and improve volumetric indices based on our previous studies (Nash, Bilsker et al. 1991, West, Crawford et al. 2014), which was accomplished. We also expected that SWIM intervention would fail to prevent cardiac decline since SWIM training was intended to mimic arm ergometry or exercise limited to the upper body. Several studies have shown that there is little difference in volumetric indices when SCI individuals are trained solely with upper body exercise (Davis,

Shephard et al. 1987, Nash and Jacobs 1998, Gates, Campbell et al. 2002, West, Campbell et al. 2012). Thus, we believe that the involvement of the lower limbs is what accounts for the different cardiac effect of the two exercise interventions. During PHLC, hind-limbs are passively rotated and therefore the blood from the paralyzed limbs is shunted back to the heart, essentially maintaining pre-load by taking the place of the disrupted venous muscle pump (Lujan and Dicarlo 2014) and decentralized vasculature. Only the forelimbs are active during SWIM training and despite the constant, but slight, pressure the water may put on the animals' trunk and lower limbs it is insufficient to aid venous return. Since, PHLC prevented reductions in LV volumetric indices despite still exhibiting impaired descending sympathetic control (i.e., reduced LFSBP and diminished hemodynamics) of sub-lesional lower vascular beds, we believe that chronic unloading is the principle cause of reduced volumetric cardiac indices in SCI.

The second major finding of this study was that the pressure generating capacity and load-independent contractile function of the heart was greatly diminished following SCI and neither PHLC nor SWIM corrected these deficits. In conjunction with reductions in LV internal diameter and reduced volumetric indices, we found that SCI animals had a marked decrease in dP/dt_{max} compared to CON animals. Although it is recognized that dP/dt_{max} changes relative to loading conditions (Kass, Yamazaki et al. 1986, Kass, Maughan et al. 1987), sympathetic stimulation plays a crucial role on basal dP/dt_{max}. Previous work has shown that following a mid-thoracic (T5) complete SCI, an injury that spares cardiac sympathetic innervation (Strack, Sawyer et al. 1988), dP/dt_{max} is relatively unaffected (Lujan, Janbaih et al. 2012); whereas with a T3 complete SCI basal dP/dt_{max} is significantly attenuated (West, Crawford et al. 2014, West, Squair et al. 2016). Despite

maintaining pre-load with PHLC, and subsequently reversing reductions in both LV internal diameter and resting EDV, dP/dt_{max} was still reduced. Furthermore, load-independent indices of contractility (Ees, PRSW and dP/dt_{max} –EDV) were also reduced in all SCI groups compared to CON. The decreased Ees, PRSW and dP/dt_{max} –EDV suggests a decline in contractile state even in the PHLC group where pre-load was maintained. This is contradictory to results from rodent models of exercise that produce athletes' heart and improve contractility with load driven training (Radovits, Olah et al. 2013). For instance, Radivotis *et al.* 2013 demonstrated that the slope of dP/dt_{max} –EDV relationship increases, shifts to the left, and contractility increases in exercise trained rats. In the SCI and SWIM group the dP/dt_{max} –EDV slope decreases and shift to the right signifying a decrease in contractility for any given volume. The PHLC group appears to decline further which we suggest is due to the larger EDV at any given dP/dt_{max}. Furthermore, at the termination of the study there was no change in LFSBP with either SWIM or PHLC. Since LFSBP is a marker of sympathetic activity to resistant vascular beds (vasomotor tone) (Baselli, Cerutti et al. 1986, Cerutti, Gustin et al. 1991, Cerutti, Barres et al. 1994, Inskip, Ramer et al. 2012), this suggests that the exercise interventions did not improve vasculature function. Taken together, the decrease in resting dP/dt_{max} , reduced load-independent measures and diminished LFSBP, implies that maintaining pre-load cannot remedy systolic dysfunction and the pressure generating deficits observed following SCI.

Next, we demonstrated that the beta agonist DOB elicited temporally different responses post-SCI and that by five weeks post-SCI drug administration normalized contractile indices and volumetric indices in all SCI groups (SCI, PHLC and SWIM). Our

adapted DOB protocol elicited the same responses as has been previously demonstrated in which there was an increase in HR and CO alongside a decrease in EDV and maintenance of SV in uninjured rats (Plante, Lachance et al. 2005). At one week post-SCI, there was very little response to DOB administration, which is in agreement with previous work in female rats with SCI (DeVeau, Martin et al. 2016). Although there was an increase in HR, there was no increase in other systolic parameters derived from echocardiography (i.e. CO and SV). There was also no change in EDV as was seen pre-SCI and in uninjured animals (Plante, Lachance et al. 2005). By five weeks post-SCI, DOB elicited a marked increase in HR, SV and CO in all SCI groups, normalizing systolic function. The degree of increase in both CO and SV at five weeks, and the lack of change in these measures at one week, suggests that there are intrinsic changes in the LV that manifest by five weeks post-SCI. At one-week post-SCI, rats are still likely in (or only just recovering from) neurogenic shock and are therefore likely to exhibit a reduced responsiveness to sympathomimetics (Guly, Bouamra et al. 2008, West, Popok et al. 2015). By five weeks post-SCI, it is likely that an adrenergic hyper-responsiveness may have developed in the heart. In clinical studies, hyper-responsiveness to norepinephrine and other alpha-mimetics have been observed post-SCI (Mathias, Frankel et al. 1976, Krum, Louis et al. 1992, Yeoh, McLachlan et al. 2004), which may be due to a compensation for lower levels of circulating catecholamine in SCI (Schmid, Huonker et al. 1998). Moreover, we have shown previously that female SCI rats are hyper-responsive to low dose Dobutamine (DeVeau, Martin et al. 2016). In addition to tracking the changing DOB response profile, we also evaluated contractile induced changes at the study termination. When DOB was administered during PV assessments, we found that

the pressure-generating capacity of the heart was improved to pre-SCI levels in all SCI groups. End systolic pressure and rates of contraction were also normalized with incrementally increasing dosages although EDP remained unchanged in all groups. Since DOB acts directly on cardiac beta-receptors (and thus bypasses disrupted sympathetic circuitry), the ability of DOB to normalize cardiac function suggests that resting inotropic dysfunction in SCI is a direct result of reduced sub-lesion sympathetic activity.

Finally, PV analysis revealed altered cardio mechanoenergetics (the efficiency of the cardiac pump function) following contusive SCI. Spinal cord injury induced an increase in the arterial elastance (Ea), which is an index of after load that takes into consideration peripheral vascular resistance and arterial compliance (Sunagawa, Maughan et al. 1983). In an uninjured system, the heart will distribute blood into the peripheral vascular tree at a particular volume and rate that matches the vascular system's ability to receive it, therefore resulting in optimal cardiac energetics (Kass and Kelly 1992). Since Ees decreases (discussed above) as Ea increases in the SCI group, the ventriculoarterial coupling ratio is thus increased, suggesting that the ability of the arterial system to receive blood distributed from the heart at a particular rate do not match. However, following the PHLC intervention, this decoupling was not observed. Although there was indeed a decrease in Ees in the PHLC group, there was no increase in Ea thus restoring the ventriculoarterial coupling ratio to CON. Several clinical studies have shown that PHLC improves femoral artery hemodynamics in individuals with SCI (Ballaz, Fusco et al. 2007, Ballaz, Fusco et al. 2008). If similar changes occur systemically to the arterial tree, then this could be expected to improve the vascular system's ability to receive blood distributed from the heart. We also found that the SVR

was improved with PHLC, further suggesting that PHLC is positively influencing peripheral vasculature. Ultimately these changes could make the peripheral vascular tree more compliant for blood redistribution and decrease afterload thus normalizing ventriculoarterial coupling.

In summary, our data provides novel insight into cardiac dysfunction after SCI and elucidates underlying causes. We confirm previous clinical findings that upper body exercise alone (SWIM) does not prevent cardiac decline, neither volumetric nor contractile measures. Since measures of contractility were also not corrected with PHLC despite improvements in volumetric indices, suggests that 1) sympathetic stimulation is vital for maintenance of normal inotropic cardiac function and 2) that there are likely intrinsic structural changes occurring in the myocardium that contribute to resting contractile dysfunction that cannot be offset without some normalization of descending sympathetic control.

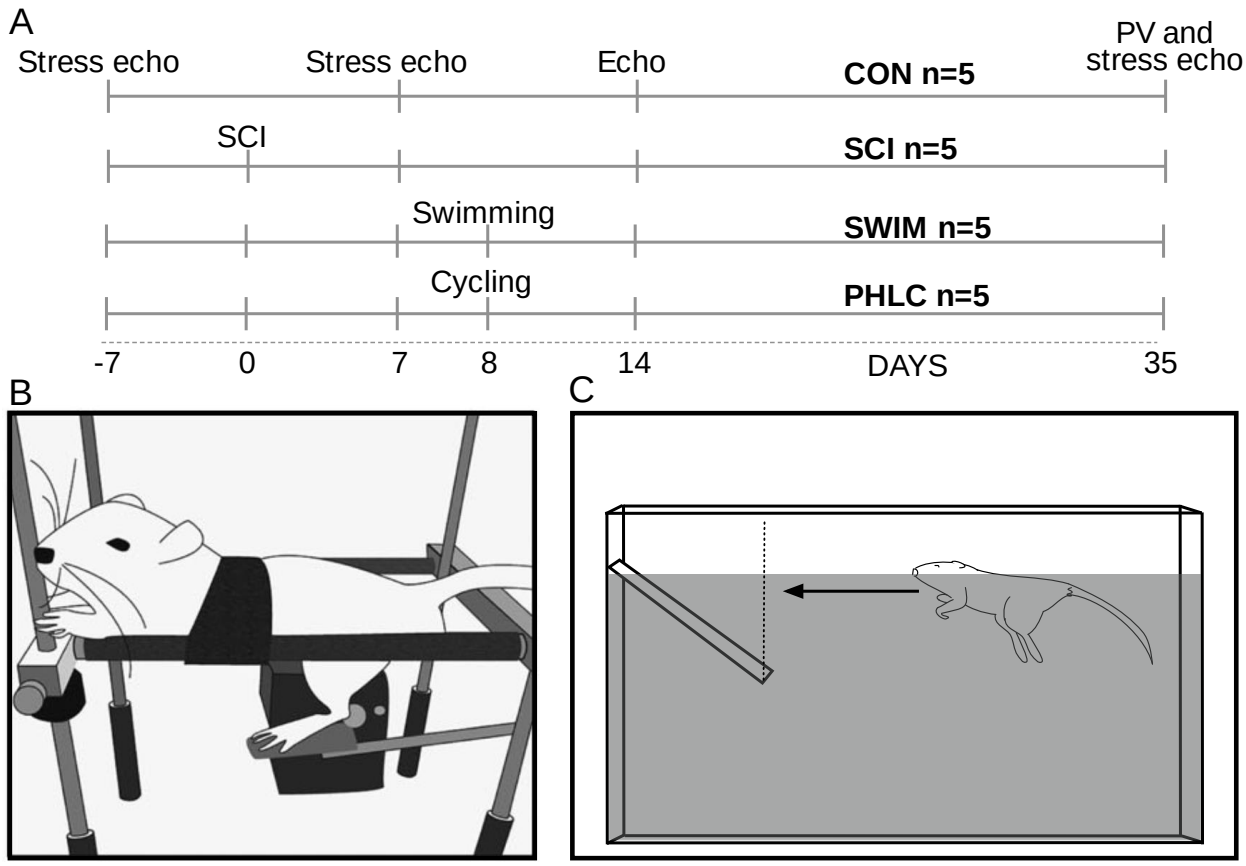
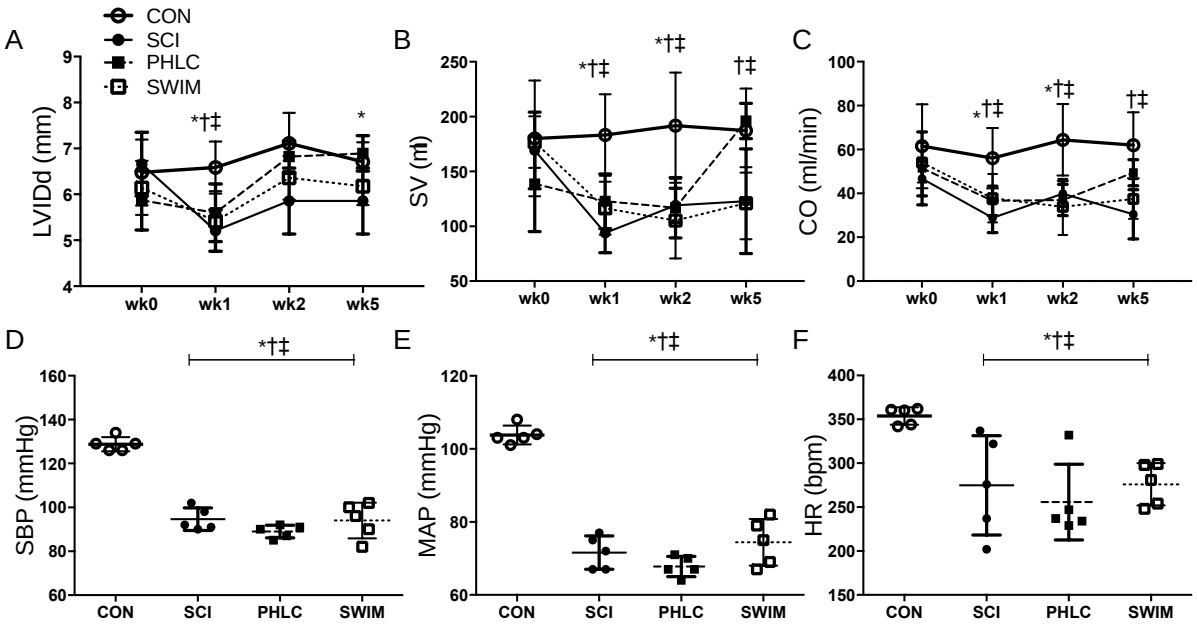


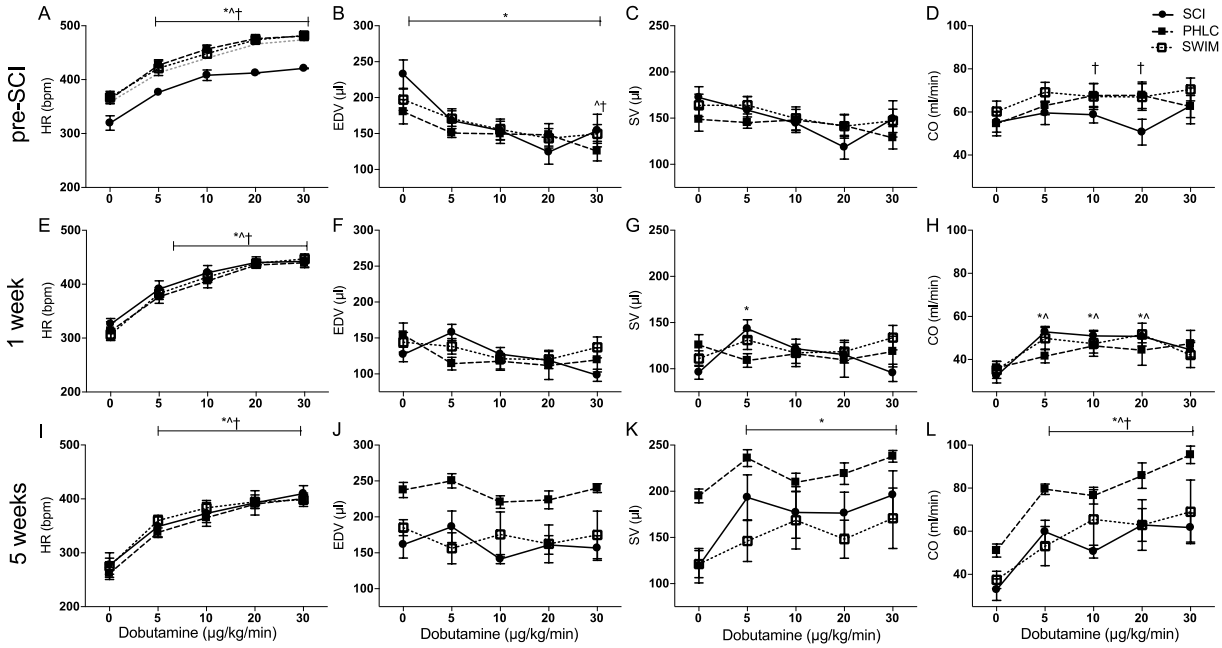
Figure 18. Overview of study design and schematics of the exercise interventions.

A. Study design. Animals were assigned to a control (CON), T2 spinal cord injury (SCI), T2 SCI with passive hind-limb cycling (PHLC), or a T2 SCI with swimming (SWIM) intervention. B. Cycling was performed for 30mins daily for 25 days. C. Swimming was also performed daily (6 x 5min sessions, 20 min break) for 25 days in a custom-made swim tank (60”length x 18”height x 7”width).



<u>Figure 19</u>. Cardiovascular function diminishes following T2 400KD spinal cord injury and PHLC appears to normalize cardiovascular dysfunction.

Left ventricle internal diameter during diastole (LVIDd; panel A.) is reduced post-SCI but brought back to control levels with PHLC and SWIM interventions. Stroke volume (SV; panel B.) and cardiac output (CO; panel C.) were only normalized with PHLC. Systolic blood pressure (SBP; panel D.) and mean arterial pressure (MAP; panel F.) were reduced after SCI and remained lower with both PHLC and SWIM. P-values represent post-hoc testing following a significant RM ANOVA main effect for echocardiography derived indices (A-C) and one-way ANOVA main effect for PV analyses (D-F). All groups n=5. Data are presented as means ± SD.*P<.05 CON vs. SCI; † P<.05 CON vs. PHLC ; ‡ P<.05 CON vs. SWIM.



<u>Figure 20</u>. Echocardiography-derived responses to the beta agonist DOB reveals a blunted response at 1 week post-SCI but hyper-responsiveness to DOB at five weeks post-SCI.

Pre-SCI response are represented by panels A-D; 1 week responses represented by panels E-H; 5 week responses represented by panels I-L. Heart rate (HR), End diastolic volume (EDV), Stroke Volume (SV) and Cardiac output (CO). P-values represent post-hoc testing following a significant RM ANOVA main effect for Dobutamine concentration. All groups n=5. Data are represented as means ± SD.* P<.05 for SCI at that dose vs. 0µg; ^ P<.05 for SWIM at that dose vs. 0µg; † P<.05 for PHLC at that dose vs. 0µg.

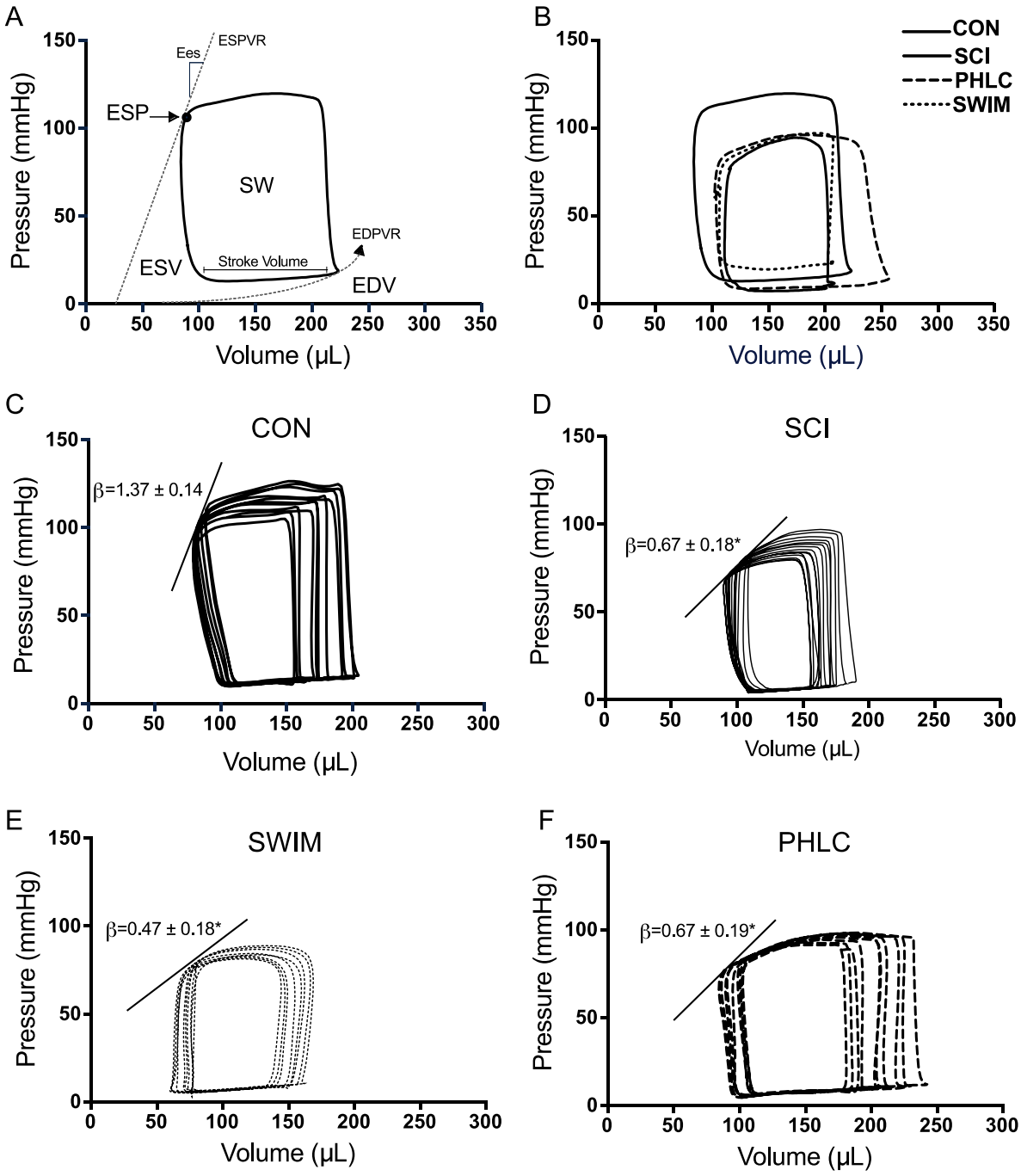
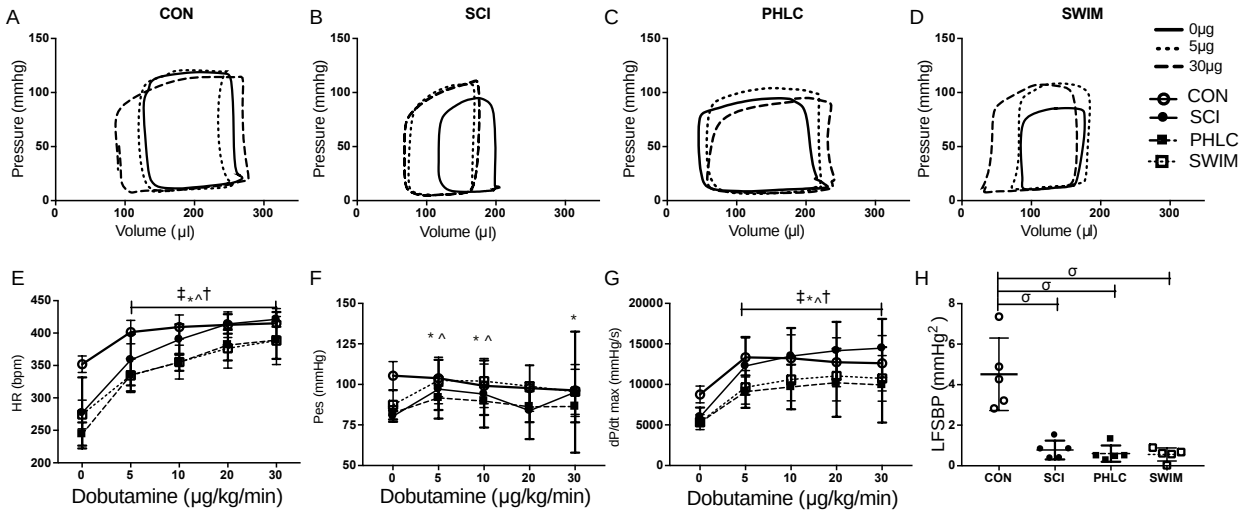


Figure 21. Terminal pressure-volume assessments demonstrate reduced basal pressure generating capacity and load-independent metrics in all groups compared to CON.

A. Representative loop from CON labeled with relevant measurements acquired from PV analysis. B. Representative loop from one animal in each group demonstrating diminished volumetric and pressure indices in SCI and the recovery of volumetric indices in PHLC. Each loop represents 15 cardiac cycles averaged together. C-F. Inferior vena cava occlusions from each group representing the load-independent measures acquired. Note that all SCI groups exhibited a significantly reduced Ees beta-coefficient. Load-independent metrics were analyzed with mixed model regression analysis and P-values represent post-hoc testing following a significant main effect for group. All groups n=5. Data are represented as means ± SEM. * P<.05 vs. CON. End systolic pressure, ESP; End systolic volume, ESV; end diastolic volume, EDV; End diastolic pressure-volume relationship, EDPVR; Stroke work, SW; End systolic pressure-volume relationship, ESPVR; slope of the ESPVR, Ees.



<u>Figure 22</u>. Pressure-volume derived indices in response to administration of increasing concentrations of Dobutamine.

A-D Representative P-V loops obtained from one animal in each group. Each loop contains fifteen cardiac cycles averaged together to demonstrate the increase in both volume and pressure indices in response to DOB. Dobutamine elicits increases in heart rate (HR, panel E), transient increases in end systolic pressure (Pes, panel F) and normalizes rates of contraction (dP/dTmax, panel G). Low frequency systolic blood pressure is diminished in SCI and is not influenced by exercise (LFSBP, panel H). P-values represent significant post-hoc testing following a significant RM ANOVA main effect for DOB (E-G), or group (H). All groups n=5. Data are represented as means ± SD. ‡ P<.05 for CON at that dose vs. 0µg; * P<.05 for SCI at that dose vs. 0µg; ^ P<.05 for SWIM at that dose vs. 0µg; † P<.05 for PHLC at that dose vs. 0µg. LFSBP σ P<.001 vs. CON.

Table 4. Terminal anatomical, echocardiographic and hemodynamic data from CON, SCI, PHLC and SWIM

	CON	SCI	PHLC	SWIM
Anatomical Data				
Body mass (g)	444 ± 23	314 ± 24*	316 ± 17*	309 ± 27*
Heart Mass (g)	1.48 ± 0.24	0.96 ± 0.09*	1.07 ± 0.05*	0.97 ± 0.16*
Femur Length (cm)	1.52 ± 0.02	1.46 ± 0.05	1.46 ± 0.07	1.45 ± 0.03
Hemodynamic Data				
SBP (mmHg)	129 ± 3	95 ± 5*	89 ± 2*	94 ± 8*
DBP (mmHg)	91 ± 2	60 ± 6*	57 ± 4*	65 ± 7*
MAP (mmHg)	104 ± 2	72 ± 5*	68 ± 3*	75 ± 6*
HR (BPM)	354 ± 10	275 ± 57*	256 ± 43*	276 ± 24*
SVR ((mmHg·min)/µl)	16 ± 3	30 ± 8*†	17 ± 3^‡	33 ± 16*†
LFSBP	4.51 ± 1.78	0.78 ± 0.46*	0.60 ± 0.40*	0.56 ± 0.32*
Echocardiographic Data				
Dimensions and volumes				
LVIDd (mm)	7.22 ± 0.71	5.25 ± 0.90*†‡	7.51 ± 0.60^	6.95 ± 0.60^
LVIDs (mm)	3.43 ± 0.49	3.08 ± 0.40	3.83 ± 0.73	3.45 ± 0.64
LVPWd	2.20 ± 0.36	2.15 ± 0.42	1.81 ± 0.19	1.91 ± 0.34
RWT	0.67 ± 0.15	0.80 ± 0.20	0.53 ± 0.06	0.63 ± 0.14
EDV (μl)	250 ± 43	144 ± 45*†‡	267 ± 37^	217 ± 27^
ESV (μl)	45 ± 17	41 ± 11	56 ± 24	43 ± 19
Systolic Function				
SV (μl)	205 ± 44	102 ± 40*†	211 ± 18^	174 ± 40*†
EF (%)	89 ± 10	67 ± 11*†‡	86 ± 6^	89 ± 15^
CO (ml/min)	67 ± 16	29 ± 12*†	55 ± 6^^	49 ± 13*
Diastolic Function				
E (mm/s)	0.85 ± 0.07	0.76 ± 0.10	1.40 ± 0.97	0.85 ± 0.08
Pressure Volume Data				
Systolic Function				
SW (mmHg·mL)	18 ± 3	7 ± 2*	12 ± 2*^‡	6 ± 2*†

Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate; BPM: beats per minute; SVR: systemic vascular resistance; LFSBP: low frequency systolic blood pressure; LVIDd: left ventricular internal diameter during diastole; LVIDs: left ventricular internal diameter during systole; LVPWd: left ventricular posterior wall thickness during diastole; RWT: relative wall

thickness; EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume; EF: ejection fraction; CO: cardiac output; E: transmitral filling velocities during early diastole; SW: stroke work; SWI: stroke work index; CI: cardiac index; Pes: end systolic pressure; Ea: arterial elastance; Ees: end systolic pressure volume relationship; PRSW: preload recruitable stroke work; Ped: end diastolic pressure; EDPVR: end diastolic pressure volume relationship. P-values represent *post-hoc* testing following a significant one-way ANOVA. All groups n=5. Data are displayed as mean ± SD. *P< 0.05 vs. CON; ^P<0.05 vs. SCI; †P< 0.05 vs. PHLC; ‡ P<0.05 vs. SWIM. #Values were derived from mixed model linear regression and not from averaged individual animal data, error represented as standard error of the mean for these metrics.

CHAPTER VI

PRELIMINARY DATA: LIMITING ACTIVITY AFTER AN INCOMPLETE SPINAL CORD INJURY EXACERBATES CARDIAC DYSFUNCTION

Introduction

It is widely accepted that high-level (T6 and above) spinal cord injury (SCI) is associated with substantial cardiac dysfunction. From clinical and pre-clinical studies, instances of cardiac atrophy,(Kessler, Pina et al. 1986, Nash, Bilsker et al. 1991) systolic dysfunction(Kessler, Pina et al. 1986, West, Bellantoni et al. 2013) altered hemodynamics(Wecht, Zhu et al. 2013) and increased bouts of arrhythmias (Lujan, Palani et al. 2010, Lujan, Janbaih et al. 2012) have all been documented chronically following SCI. Furthermore, the level and the autonomic completeness of the injury appear to correlate with the degree of chronic cardiac dysfunction observed (West, Mills et al. 2012). Yet it has, thus far, been difficult to separate the relative contributions of the decentralized autonomic nervous system (ANS) and the immediate and profound inactivity to observed chronic cardiac abnormalities. It is evident that the injury itself plays a crucial role on basal cardiac homeostasis. For instance tetraplegic patients have lower resting heart rates (HR) than their able-bodied and paraplegic counterparts (Zhu, Galea et al. 2013). However, prolonged cardiac unloading (i.e. immobility and inactivity) can also lead to substantial cardiac remodeling. It has been documented in able-bodied population that long periods of bed rest can reduce left ventricle internal

diameter (LVID) and systolic function (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Perhonen, Zuckerman et al. 2001).

There is profound and extensive immobility following initial SCI. Patients are generally in intensive care for many weeks and a large portion of individuals also sustain additional injuries, which makes implementing therapies complicated. Thus, SCI patients are bedridden and immobile acutely and for long periods of time, ultimately un-loading the heart. Furthermore, it has been suggested that early morbidity and mortality in the SCI population is a result of inactivity related illnesses such as cardiovascular disease (Garshick, Kelley et al. 2005). Exercise can be prescribed as means to offset cardiovascular disease and cardiac decline. Exercise regimens applied chronically such as functional electrical stimulation have been shown to reverse cardiac atrophy (Nash, Bilsker et al. 1991) and in one case study active-arm passive-leg exercise improved cardiac flow indices (West, Currie et al. 2015) To date, only one pre-clinical study has looked at the effects of implementing an acutely applied exercise intervention, passive hind-limb cycling, and has demonstrated that cardiac decline can be prevented (West, Crawford et al. 2014). Although exercise interventions provide beneficial effects on cardiac function there is no general consensus on the type, longevity, duration or time of implementation (i.e. acute vs. chronic). It is well established that appropriately timed therapies have the potential to offset locomotor and sensory (central nervous system; CNS) deficits. Therefore it is not unreasonable to think that the ANS may respond in a similar manner.

Modest increases in physical activity (PA) acutely, may help prevent maladaptive cardiac remodeling. It is well accepted that PA is associated with a myriad of positive

effects and that increasing PA supports a healthy life-style. Guidelines for PA in the adult SCI community have only recently been established, most likely due to the heterogeneity of this population (Ginis, Hicks et al. 2011). It maybe the case that increasing the level of activity, even that associated with daily living, early post-injury could induce beneficial cardiac changes along the lines of those seen with exercise. To our knowledge, there has been no pre-clinical study that gauges the effects of modest amounts of activity on the recovery of cardiac function following incomplete SCI. Understanding how limiting the amount of activity, such as confinement to a wheelchair, can effect cardiovascular function post-SCI would benefit the SCI community and potentially influence how therapies are tailored and when they are implemented. It could very well be the case that several therapies are needed to target the locomotor and sensory system and the autonomic individually.

The goal of this study was to investigate the effects of changing "spontaneous" activity on cardiac function by altering (increasing of decreasing) the space available for animals to locomote. Housing animals in tiny cages (TC) attempts to mimic the effects of confining individuals to wheelchairs and bed-rest while large cages (LC) represent an SCI cohort with increased daily activity. Cardiac function was tracked over time with *in vivo* echocardiography and correlated with bi-weekly assessments of overnight activity and 3D kinematics.

Methods

Experimental Design

Experiments were conducted on 39 female Sprague Dawley (SD) rats (age = 15 wks, weight 250-260g). All procedures were approved by the University of Louisville Animal Care and Use Committee. Animals were randomly assigned to either an uninjured control (CON n=7), a T2 25 g-cm SCI housed in tiny cages (TC n=16) or a T2 25 g-cm SCI housed in large cages (LC n=16). Cardiac function was assessed prior to SCI, week one post-SCI and then every two weeks for ten weeks post-SCI. The distance animals traveled during their most active hours (overnight activity) was recorded at weeks one and two post-SCI and then every two weeks post-SCI for ten weeks. Hind-limb function during overground locomotion was assessed weekly using the BBB Open Field Locomotor assessment(Basso, Beattie et al. 1995) and with kinematics(Caudle, Atkinson et al. 2014) at one, two and ten weeks post-SCI. At the study termination a cohort of animals from each group was selected based on their BBB scores for SC histological assessments (LC & TC n=10) and hearts were collected from all animals. One animal was eliminated from the TC group because it regained overground stepping such that its BBB >16.

Spinal Cord Injury Surgery

Animals were anesthetized with a ketamine/xylazine/acepromazine cocktail (50/0.024/0.005 mg/kg i.p.) and a dorsal mid-line incision was made through the skin and musculature overlying the C8 to T4 spinal segments. A laminectomy was performed at T1 and T2 to expose the underlying T2 spinal cord and moderately-severe injury (25g-cm) was delivered with a MASCIS Impactor (Rutgers University, NJ). All animals were then given Buprenorphine (0.1 mg/kg, SC) twice a day for three days, gentamycin

(Gentamicin sulfate 15 mg/kg SC) once a day for seven days, and 5mL of Lactated Ringers for five days and as needed for hydration. Bladders were expressed manually for seven days or until spontaneous voiding returned. For one week following injury, animals were under dietary restrictions to fruit, spinach and nutritional supplements (76A). Animals were slowly acclimated back onto dry food. Three days after injuries, animals were placed into their housing conditions (tiny or large cages). Animals were housed socially, two per cage, on the same 12-hour light/dark cycle.

Housing Conditions and Overnight Activity

Animals were placed into their respective housing conditions three days post-SCI. Tiny cages dimensions (7.5 x 8.5" x 8") and large cages dimensions (14" x 18" x 8") (Fig.23). Holes were drilled along the edges of walls for ventilation (TC 24, LC 32). All cages were fitted with a clear plastic lid. Animals were still housed socially despite confided spaces. All animals were housed on alpha dri throughout the study. Bedding was changed daily for the tiny cage group and every two days for the large cage group. Overnight activity was recorded at weeks one, two and every other week following for ten weeks. Each animal was marked with a 1" diameter black dot on her back, between the iliac crests. Cameras (acA645-100gm Area Scan Camera; Basler, Ahrensburg, Germany) were placed above each cage so that the entire bottom of the cage and the animals' movements were recorded. One camera could either capture one large cage or two small cages simultaneously. Activity was recorded from 6pm to 6am (12 hours). One minute was captured every ten minutes within each hour so that there were 6min recorded for each of the 12 hours (72minutes total).

Echocardiography

Echocardiographic assessments were acquired at pre-SCI, one and two weeks post-SCI and then every other week until ten weeks post-SCI. Animals were prepped for assessments and images were captured according to our labs protocol and as previously described.(West, Crawford et al. 2014) Briefly, respiration was monitored with induction plethysmography, heart rate (HR) was monitored with a four lead ECG pad (Visual Sonics, Toronto, CA) and core body temperature was maintained at 37-38°C. To assess systolic function, images were captured along the parasternal short-axis and five consecutive cardiac cycles during expiration were averaged. Images captured in the apical-four chamber view were used to evaluate diastolic function.

Kinematic Assessment of Stepping

At week one, two and ten following injury, hindlimb joint range of motion during stepping for each group was assessed with 3D kinematics as previously described.(Kuerzi, Brown et al. 2010), (Magnuson, Smith et al. 2009) Briefly the hind limbs were marked on the iliac crest (I), hip (H), ankle (A) and toe (T). Tracking the movements of these markers allows us to define movements of the limb by three distances (I-H, H-A, A-T) and two angles (I-H-A & H-A-T). As the animal moves from one end of the tank to the other, four passes, where all points are clearly visible, were analyzed using MaxTraq3D (Innovision Systems, Columbiaville, Michigan). The excursion of each step was averaged within in each group and compared between groups

and across time. Paw placement and stride length was assessed with bottom camera and compared between groups.

Histology

Animals were perfused with phosphate buffered saline (PBS) via cannulation of the ascending aorta to avoid damage to the cardiac tissue. The heart was then cleaned of excess fat and vessels and was weighed before being placed in 4% PFA. After 24 hours in fixative, hearts were cryoprotected in 30% sucrose with sodium azide for two days and then blocked in cryoprotective media. The entire spinal column was also extracted and placed in 4% PFA for one day. The SC was then removed from the column and cryoprotected in 30% sucrose for at least 48 hours.

Hearts were sectioned at 10µm and processed for collagen deposition with conventional Masson's Trichrome stain. Images were captured at 20X magnification along the left ventricle free wall from five different sections at least 70µm apart using consistent camera settings. Collagen deposition was quantified as a percent of the total area ($40\mu m^2$) of the image. Specifically, images were set to a threshold to identify collagen-positive tissue and the area of collagen-positive tissue was divided by the total area of the image (in pixels). The percent of collagen was averaged across the five images captured per animals to obtain one data point per animal.

Spinal Cords (SC) were sectioned through the epicenter at 30µm. Sections were allowed to air dry for 30min before storing at 4°C overnight. The following day, SC sections were warmed for 20min and processed for spared white matter using Eriochrome Cyanin (EC) stain as described previously (Smith, Burke et al. 2006).

Statistical Analyses

Statistical analyses were performed with SPSS (v22) and the level of significance was se at $P<0.05$. All echocardiographic assessments were analyzed repeated-measures analysis of variance (RM ANOVA) with one for within group (i.e. over time) and between group comparisons. Parametric ANOVA assumptions were tested (normality and Mauchly's sphericity test). The Greenhouse-Geisser correction was used to adjust degrees of freedom and correct the p value when the variance test was significant revealing unequal variance. All significant main or interaction ANOVA effects were further investigated using Tukey's HSD *post-hoc* testing. Data are represented as mean ± standard deviation.

Results

First, we tracked the temporal cardiovascular changes post-SCI using *in vivo* echocardiography. We demonstrated that both groups had similar cardiac function pre-SCI (all $p=1.0$). At one week post-SCI both the TC and LC groups had a similar drop in chamber dimensions and cardiac flow indices (Fig.24 A-D). We found that SCI induced changes in LVID that persisted at ten weeks despite caging size (Fig.24A $P<0.05$ TC and LC vs. pre-SCI). End diastolic volume and cardiac flow indices were also diminished compared to pre-SCI levels in both groups (Fig.24B $P<0.05$). Flow indices, stroke volume (SV) and cardiac output (CO) were also reduced compared to pre-SCI levels (Fig.24C-D all $P<0.05$). Interestingly there was a significant interaction effect for both volumetric and flow indices. The EDV was significantly different between groups at

eight and ten weeks (Fig.24B $P<0.05$). There was also a group difference in SV at weeks six and eight (Fig.24C $P<0.05$). Although this difference did not persist at ten weeks post-SCI it was approaching significance ($P=.057$).

The animals' ability to step overground was evaluated with the open field locomotor assessment (BBB) weekly. At one week post-SCI both groups BBB score decreased significantly from baseline (Fig.25A, all $P<.0001$; LC average BBB 9 ± 2; TC average BBB 8 ± 1). Both groups locomotor function plateaued by three weeks post-SCI and were not significantly different from one another (Fig.25A, LC average BBB 10 ± 4; TC average BBB 10 ± 1). At ten weeks post-SCI, there was no group difference in BBB scores (Fig.25A,both average BBB=10). Throughout the study overnight activity (ONA) was evaluated for between group comparisons. Every two week post injury ONA was recorded and quantified as the distance animals traveled overnight in meters. There was a significant difference in the distance traveled between TC and LC groups at all time points post-SCI (Fig.25 all, $p<.0001$). The TC animals averaged 42m per night each week while the LC animals averaged 155.3m per-night. Surprisingly, EDV and SV did not correlate with ONA (Fig.25C&D). However, there did appear to be a trend such that in the TC group animals covering more distance overnight had higher EDV and SV. The trend was not similar in the large cage group.

At the termination of the study spinal cords (SC) and hearts were collected for histological analyses. Spared WM was similar for the LC (5.07 ± 3.17) and TC (4.78 ± 1.62) groups (Fig.26A&B). There was also no significant difference in the amount of collagen deposition in the left ventricular free wall between groups (data not shown).

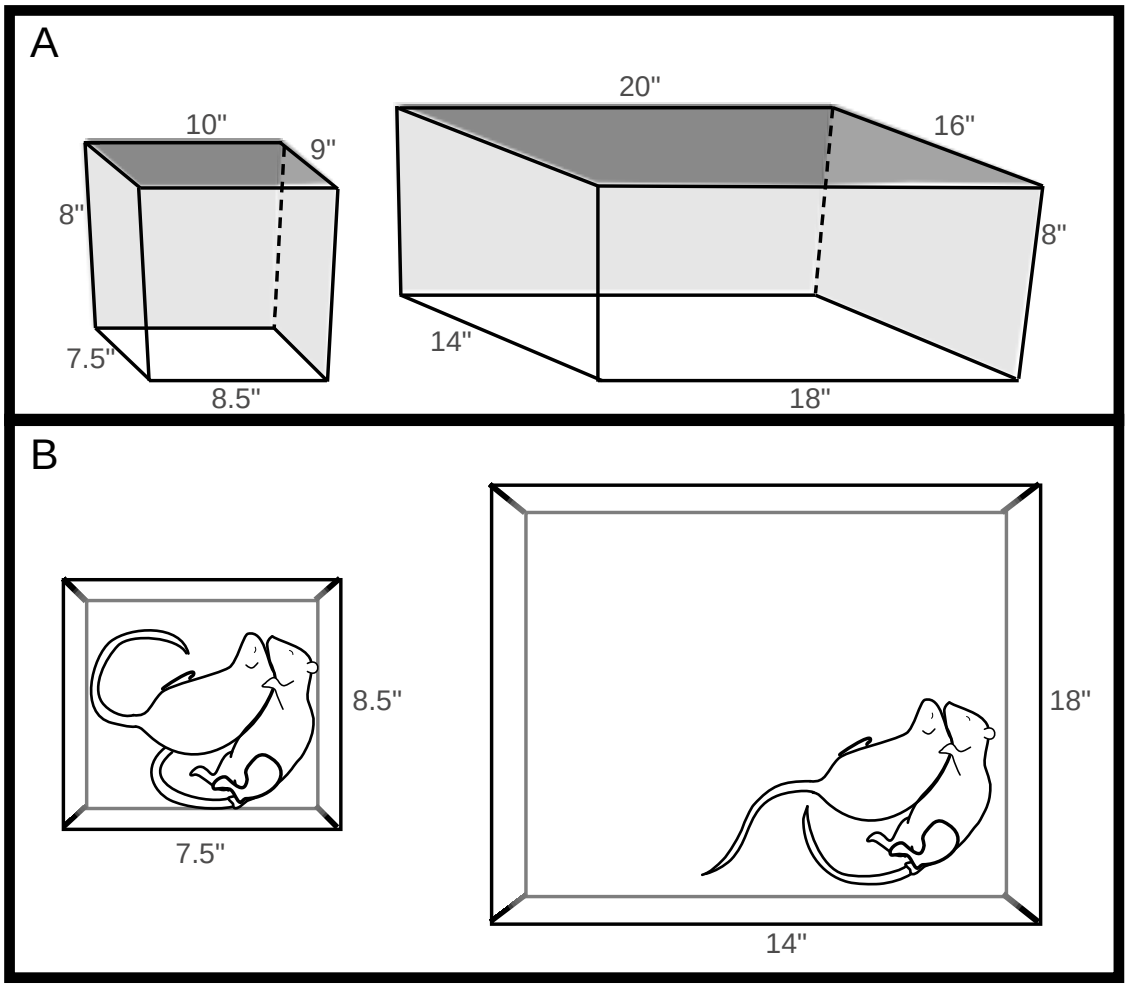
Preliminary Discussion

This is the first report of further impairment in basal cardiac function due to limiting the amount of activity rodents are able to generate. We demonstrate that with a T2 25g-cm injury cardiac dysfunction is sustained ten weeks post-injury despite housing conditions. We then show that there are different trends in flow-derived indices when correlated to ONA between the housing conditions. Finally, at the termination of the study we confirm that there are no differences in the injury itself defined by the white matter spared and that there were no differences in left ventricle characteristics.

We first show that basal level of activity plays an integral role in the resting cardiac function following SCI. By week one post injury there was an initial drop in both LC and TC animal cardiac function. We believe that this initial drop in cardiac function is verification of the presence of neurogenic shock which is characterized by marked reduction in blood pressure and HR (Krassioukov, Warburton et al. 2009). Persistent hypotension and bradycardia would directly influence flow derived cardiac indices such as CO and SV. All animals recovered from neurogenic shock by week two with a similar degree of cardiac recovery. However, by week four there was a further reduction in cardiac function within the TC group. Although the difference was not statistically different between groups there is a noticeable fall in flow-derived indices. Function then remained lower in the TC group such that at week six and eight there are statistically significant differences between the groups in cardiac function and EDV at the eight and ten week time points. This drop at four weeks was not surprising to us since reductions in SV have been noted to occur as early two weeks following forced bed rest and spaceflight (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Perhonen,

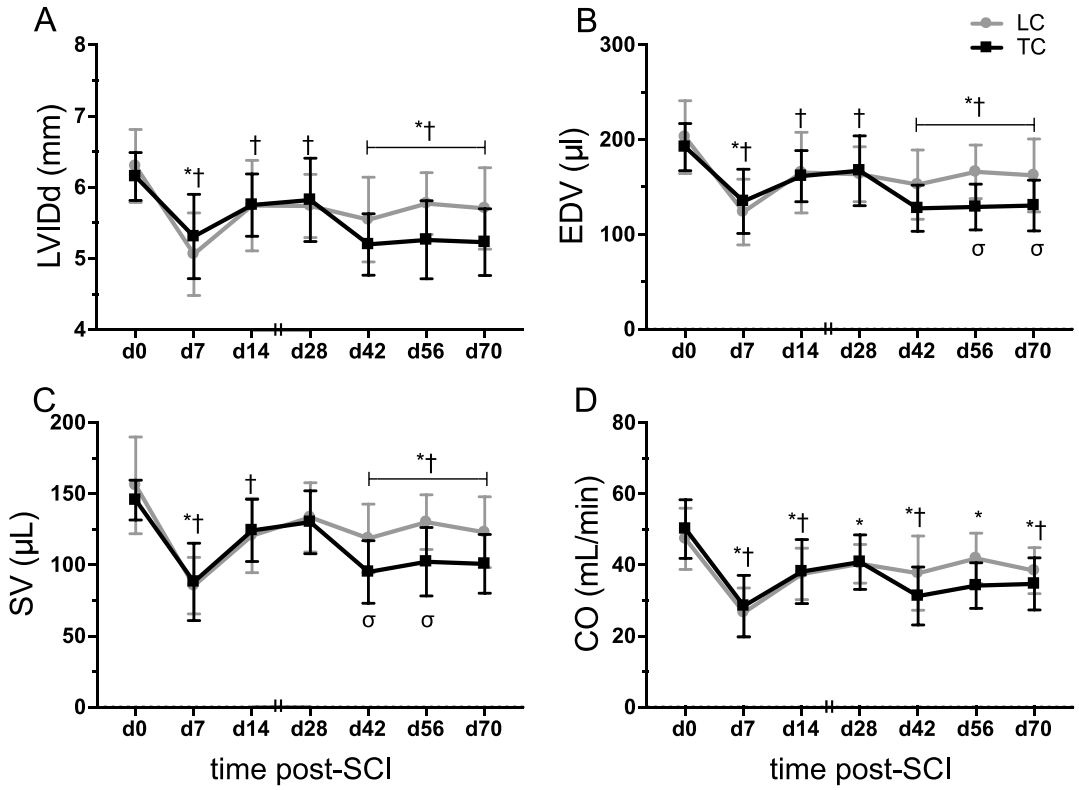
Zuckerman et al. 2001). However, we did not expect the TC animals to recover to the same degree following neurogenic shock at the two week mark. Considering the difference in the distance covered during their most active hours we expected the effects of these differences to manifest earlier.

Although there was a considerable difference in the amount of ONA generated between groups, there was not a significant correlation between ONA and cardiac metrics. Since animals with more space to move around would have the ability to maintain pre-load to the heart, we suspected there would be a positive correlation between ONA and cardiac metrics. We therefore correlated ONA to flow derived indices EDV and SV however there were no significant correlations. There appeared to be a trend in the TC group such that animals that traveled more distance overnight had larger EDV but the correlation was not significant. In addition to this the LC group had an opposite relationship, where several animals with the lowest amount of activity had the highest EDV and SV. This suggests to us that the basal amounts of activity influence cardiac function only to a certain degree creating a ceiling effect such that animals in the LC cardiac function could not recover beyond a certain point due to the injury.



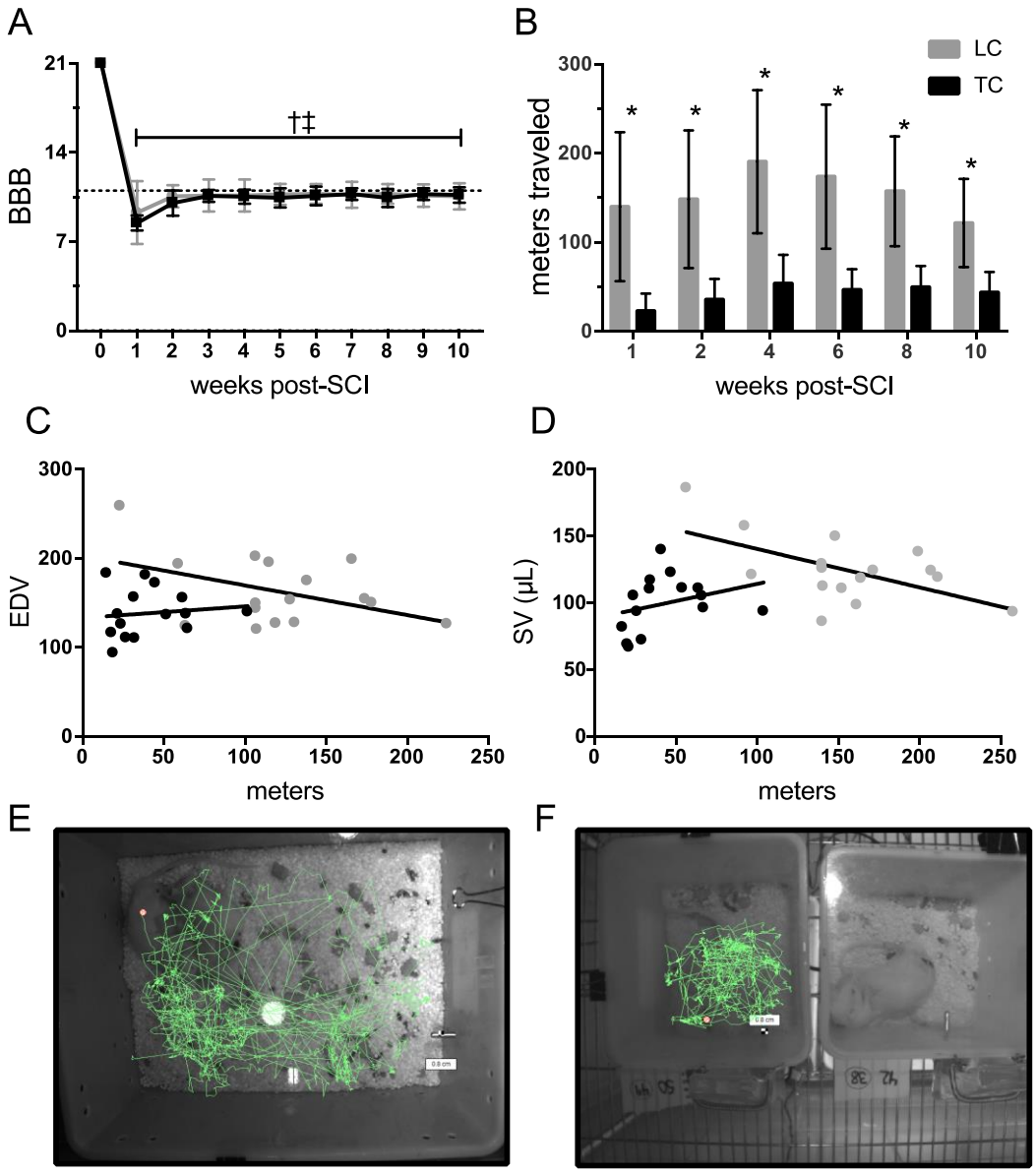
<u>Figure 23</u>. Cage dimension schematic demonstrating the difference in cage size and space available for movement.

A. Cage dimensions. B. Scaled representative schematic of the space available for locomotion.



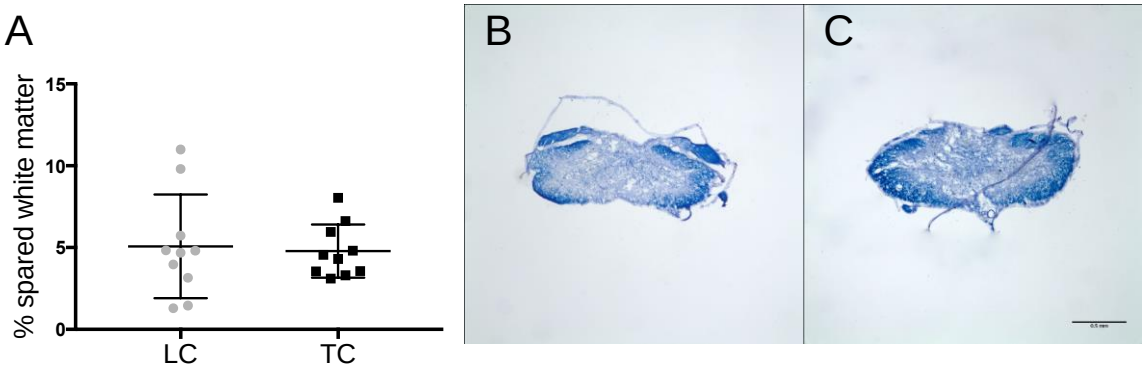
<u>Figure 24</u>. Cardiovascular function following a T2 25g-cm SCI is diminished at one-week post-injury and, although some recovery of function does occur, flow indices remain attenuated at ten weeks post-SCI despite housing condition.

A. Left ventricle internal diameter during diastole (LVIDd). B. End diastolic volume (EDV). C Stroke volume (SV). D. Cardiac output (CO). *P<0.05 TC vs. pre-SCI. † LC vs. pre-SCI. σ P<0.05 TC vs. LC.



<u>Figure 25</u>. The distance rodents travel over night (ONA) does not affect their overground step nor does it correlate to end diastolic volume or stroke volume.

A, locomotor assessment BBB. B, Group comparison of overnight activity. C, Neither end diastolic volume (EDV; panel C) nor stroke volume (SV; panel D) had a significant correlation with distance traveled overnight. E&F, representative images of the ONA tracked with custom-made MaxTRAQ software. *P<0.0001 LC vs. TC; †P<0.05 LC vs. pre-SCI; ‡ P<0.05 TC vs. pre-SCI.



<u>Figure 26</u>. The white matter spared did not differ between the large cage (LC) and tine cages (TC) groups.

The average white matter spared for the LC was 5.07 ± 3.17 and TC was 4.78 ± 1.62.

Table 5. Week 10 anatomical and echocardiographic data from animals housed in large and tiny cages

	LC n=16	TC n=16
Anatomical data		
Body weight (g)	269 ± 13	273 ± 14
Heart weight (g)	1.02 ± 0.09	0.96 ± 0.06
Heart weight/femur	2.84 ± 1.48	2.63 ± 0.16
Collagen Content	0.00193 ± 0.0009	0.00185 ± 0.0006
Echocardiographic data		
Dimensions		
LVIDd (mm)	5.2 ± 0.7	4.8 ± 0.5
LVIDs (mm)	2.8 ± 0.8	2.6 ± 0.4
EDV (µl)	149 ± 38	122 ± 26
ESV (µl)	37 ± 22	29 ± 11
Systolic function		
SV (µl)	112 ± 28	92 ± 20
EF (%)	69 ± 14	70 ± 8
CO (ml/min)	35 ± 7	32 ± 7
Diastolic function		
E (cm s-1)	690 ± 136	726 ± 107

LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; CO, cardiac output; E, peak transmitral filling velocity during early diastole; LC, large cage housed animals; TC, tiny cage housed animals; Data are displayed as mean ±SD

CHAPTER VII

CONCLUDING DISCUSSION

The above studies extend our understanding of SCI induced cardiac dysfunction, how it manifests over time and highlights the limitations of exercise interventions to correct cardiac decline. We first verify that a commonly used contusion SCI associated with a degree of regained overground stepping also induces significant and sustained systolic dysfunction chronically. We further demonstrate the importance of descending sympatho-excitatory control on basal cardiac function. We then compare the cardiac effects elicited from two commonly used exercise interventions and show that neither provide an appropriate stimulus to correct deficits in the cardiac pressure generating capability and load independent metrics of contractility. Finally, we investigate the role of increasing modest amount of spontaneous activity on cardiac function and find that limiting a rodent's basal level of activity further impairs cardiac function.

First we characterize the cardiac function following a 25g-cm contusive SCI and reveal significantly reduced function chronically. Only one other model of contusion SCI has demonstrated cardiac dysfunction at six weeks post injury. In a set of experiments conducted by Squair et al 2015, the cardiovascular dysfunction developed from several graded injury severities administered at the T2 spinal segment were compared. Of the injuries, only the most severe injury (400kdyn with 5 second dwell) resulted in chronic systolic dysfunction and altered hemodynamics (Squair, West et al. 2016). It is important

to note that terminal BBB for the severe group was 2.3 ±1.9 and the spared WM was 3-4%. There was also significant spread from the injury itself that spans roughly 2.5cm rostral and caudal beyond the epicenter. The extensive spread of this injury further disrupts sympathetic innervation from spinal segments adjacent to the epicenter. This incredibly severe injury mimics a transection more than a contusion. In our set of experiments, the contusion severities utilized allowed for some degree of overground stepping to be regained. This was critical due to the prevalence of incomplete injuries that present to the clinic which have some degree of locomotor and/or sensory function intact.

Surprisingly, there was essentially no systolic cardiac dysfunction following the lowest grade of contusion SCI (T3-MILD). This was unexpected given that overground stepping improved the least in this group (BBB average 8 ± 3) and that all the injuries administered were within the levels that supply sympathetic innervation to the heart and upper-body vasculature. However, when considering the spread of the injury and the location of the cavity generated from the injury, descending vasomotor control from the RVLM was not significantly disrupted with the mild injury. Therefore the other three spinal segments contributing sympathetic innervation to the cardiac contractile tissue and upper-body vasculature still receive supraspinal control. In addition to this, we found no evidence of increased sympathetic aborization within cardiac contractile tissue, which suggests that there was no sprouting of postganglionic neurons. This is contradictory to previous reports of increased sympathetic fiber density in contractile tissue of the myocardium in response to a T5 complete SC transection (Lujan and DiCarlo 2007). A possible explanation of these contradictory results is a difference in the injury mechanism itself. With a transection model, the entirety of supraspinal control is disrupted (Kalincik,

Choi et al. 2010). Whereas, due to the formation of the injury cavity and the spread produced by the NYU impactor, the most lateral portions of the SC remain relatively intact and myelinated (based on WM spared quantified as darkly stained compacted myelin). Dorsal hemi-sections may prove to be the preferred injury for CV geared investigations moving forward since these injuries would directly disrupt the descending vasomotor control but also leave motor control intact.

We then utilized the T2-MOD injuries to establish the critical role of sympathetic stimulation on resting systolic function. Utilizing a beta-1 specific agonist, Dobutamine (DOB), to by-pass the disrupted circuitry we demonstrate for the first time, that systolic function of a spinalized heart can be brought back to pre-injury levels with increased sympathetic stimulation. It is well established from pre-clinical studies that SCI results in lower resting levels of circulating catecholamines, noradelnaline (epinephrine) and adrenaline (Mathias, Christensen et al. 1975, Mathias, Frankel et al. 1976, Schmid, Halle et al. 2000). This reduction is most likely due to a loss of sympathetic preganglionic neurons and basal sympathetic activity from the injury. Furthermore, during an orthostatic stressor (Mathias, Christensen et al. 1980, Mathias 1995) or exercise (Schmid, Huonker et al. 1998) there is an inability of circulating levels to reach that of controls. With the lowest doses of DOB administration a drastic increase in both SV and CO was observed at six weeks post-SCI directly confirming the role of resting levels of catecholamines on systolic function. Furthermore since responses to DOB at six weeks post-SCI were present and drastically different from pre-injury responses it is likely that the myocardium has become hypersensitive to stimulation. This indeed has proven to be the case where there is enhanced responses of the vasculature to noradrenaline when it is

administered intravenously (Mathias, Frankel et al. 1976). As such, it is not unreasonable to presume that other receptors would likewise become hypersensitive to agonists following SCI.

From the comparison of PHLC and SWIM training, we demonstrate, for the first time, the limitations of exercise interventions to positively influence cardiac recovery following SCI. Previous pre-clinical studies have demonstrated that PHLC intervention implemented acutely post-SCI (6 days) has the capacity to prevent systolic dysfunction, collagen deposition (West, Crawford et al. 2014) and reduce the severity of AD (Popok, West et al. 2016). Thus there is growing interest in this exercise intervention. In regards to upper-limb only exercise, there is a general consensus that it is insufficient to prevent cardiac decline or improve flow derived metrics (Davis, Shephard et al. 1987, Gates, Campbell et al. 2002, West, Campbell et al. 2012). However, we were more interested in investigating the effects of PHLC and exercise limited to the upper-limbs (SWIM) on intrinsic contractile properties of the LV. Ultimately, this comparison revealed that neither training paradigm had the ability to correct cardiac pressure generating deficits. Furthermore, load independent measures were also reduced following SCI and despite intervention contractile function independent of loading scenarios could not be improved. This finding was unexpected since PHLC is supplementing for the inactive muscle pump and shunts blood from the lower limbs to the myocardium essentially maintaining stretch of the contractile tissue. However, PHLC also resulted in a further reduced dP/dt_{max} – EDV relationship. Since both heart mass and inotropic function (i.e. dP/dt_{max} and Ees) were similar between all SCI groups, the redistribution of blood to the heart with PHLC could be stretching myocytes beyond the optimum length for contraction. The exercise

comparison did confirm previous reports of improved flow-derived metrics following PHLC and further demonstrated the inability of SWIM training (upper-limb only exercise) to positively influence cardiac function. Unless the animals produce a substantial amount of cyclic hind-limb alteration during swimming, there is little cardiac benefit to this exercise intervention.

Passive hind-limb cycling improved the ventricular vascular coupling ratio (VAC), which is one of the more significant findings from this set of experiments. This suggests that as blood is distributed from heart into the vascular tree, the vascular system has the capacity to distend with the increased pressure and results in optimal mechanoenergetics. Since PHLC normalized the VAC ratio, it is most likely that the rhythmic cycling has a positive influence on the peripheral vasculature. It is well known that the vascular beds below the level of the lesion show signs of remodeling as early as three weeks post-SCI and ultimately contribute to unstable hemodynamics and CV dysfunction (De Groot, Van Kuppevelt et al. 2003, de Groot, Bleeker et al. 2006, Thijssen, De Groot et al. 2012). In the chronic SCI population, various forms of exercise have the capacity to improve hemodynamic function and reduce vascular stiffness (Thijssen, Heesterbeek et al. 2005). How the beneficial effects of PHLC are produced or manifest are unclear since data from previous studies is confounded. West et al. 2015 (West, Crawford et al. 2016) demonstrated that in isolated mesenteric arteries SCI resulted in a left ward shift of the concentration-response curve to phenylephrine (vasoconstrictor) resulting in a lower EC_{50}. When ACh concentration-response curves were generated there was a rightward shift, resulting in a higher EC_{50}. However, these changes could not be corrected with acute PHLC training. Other clinical studies show

that chronic PHLC does not improve resting blood flow but that acutely following cycling hemodynamics are improved and blood flow is increased (Ballaz, Fusco et al. 2007, Ballaz, Fusco et al. 2008). Furthermore, from the lesion level comparison (chapter III) concentration-response curves generated from isolated femoral arteries to ACh resulted in a leftward shift hence a lower EC_{50}, which contradicts West and colleagues results. However, the artery we harvested is a large conduit artery and the primary role of this type of vascular bed is to distribute blood throughout the lower limbs not necessarily to regulate BP. Smaller arteries and arterioles, especially mesenteric arteries, are responsible for BP regulation and should be the focus of future studies.

Finally, we demonstrate that limiting an animals' activity by controlling the amount of space they are given to move (locomote) appears to exacerbate cardiac function. Although we cannot say that we intentionally made animals in the large cages move around more, they were provided with a significantly larger area to locomote and thus the ONA from this group was significantly greater than TC animals throughout the study. The data generated form this set of experiments is the most relevant to clinical scenarios. Tiny cages were intended to mimic the extreme amount of activity and sedentary, wheelchair-bound lifestyle. In addition animals were placed in TC three days after injury, which also mirrors the acute immobilization following SCI. Following a similar pattern to the CV function observed from the initial characterization of the 25g-cm injury induced cardiac decline, both groups showed a substantial decrease in flow derived metrics one week post-SCI. This initial drop is most likely due to neurogenic shock where individuals are plagued with constant, severe hypotension and bradycardia. At two weeks post-SCI, both groups appeared to recover to a new basal level of cardiac

function. However, by four weeks post-SCI the flow derived indices dropped again in the TC group. Although the reduction did not result in a significant between group difference, the decline was obvious. It is interesting that the difference in function took roughly four weeks to manifest. We did not collect data during week three, which may have been the initial separation and would have let us visualize a gradual decrease rather than a stark separation from week two to week four. However, it is still peculiar that at week two the animals from each group appeared to recover to the same extent despite a significant difference in ONA at this time point. This pattern of recovery hails to similarities with studies where immobilization and space flight result in reductions in the LV internal diameter and systolic function (Levine, Zuckerman et al. 1997, Perhonen, Franco et al. 2001, Perhonen, Zuckerman et al. 2001). Animals housed in the tiny cages would ultimately lead to further cardiac unloading just as forced bed rest and immobilization would, since they are not moving about the cage and shunting blood back to the myocardium. Animals housed in the large cages had significantly more ONA thus most likely maintaining cardiac pre-load. It would be interesting to see if the cardiac function of TC animals could be brought back to LC or even pre-injury levels with an exercise intervention.

In summary, the above studies extend our knowledge of SCI induced cardiac decline, shed light on the limitations of exercise and ultimately reveal the necessity of controlling overnight activity when studying CV function following SCI. Utilizing a severe contusion SCI has proven to be the most beneficial for investigating CV function, since the more severe injuries appear to sufficiently disrupt descending vasomotor control. Since CV decline is affected by both the initial insult and then the subsequent

extreme inactivity, it has previously been difficult to separate the relative contributions of either. From the DOB experiments we showed the critical role of sympatho-excitatory control on resting cardiac function. Both SV and CO increased substantially at six weeks post-SCI with administration of the beta agonist that bypasses disrupted circuitry. Then we show that limiting animal movement further exacerbates cardiac decline demonstrating the egregious effects of inactivity on cardiac function following injury. Ultimately it is a combination of the two, the injury and inactivity, which result in chronic cardiac function. We currently have the means to remedy one—inactivity. Moving forward, custom-made wheelchairs and housing conditions that limit ONA and cardiac pre-load should always be used when evaluating SCI induced cardiac decline. We will then be able to control effects of inactivity and better investigate the results of applied interventions on cardiac function and the injury itself.

Potential Pitfalls

Echocardiography was the main instrument used to assess cardiac performance during these experiments, which is notorious sensitive to imaging quality, heart rate, and loading conditions and therefore has a high variability. Within the initial set of experiments each injury cohort only had a small number of animals being followed over a long period of time with echo and no other CV assessment. Therefore this limits our ability to interpret our initial data. Furthermore, from power analyses conducted, we would need at least eight animals in each group to sufficiently detect between group differences. Blood pressure measures are never acquired within any set of these experiments barring terminal measures during the exercise comparison. Blood pressure

recordings are a hallmark for CV studies and without insight to vascular changes it is difficult to understand why cardiac measures are different. However, the means to assess BP in rodents are limited and often incredibly invasive. It would be ideal to record BP simultaneously with echo measures.

From the pressure myography portion of these experiments only the femoral artery was harvested and investigated. Since this is a large conduit artery and is not necessarily a blood pressure regulator our interpretation of the data is limited. It will be important to investigate small arteries and arterioles moving forward. These arteries have been neglected in literature most likely due to the accessibility of these arteries in the clinical population. Furthermore, we only generate vasodilation response curves and neglect vasoconstriction, which limits how we can relate our data to clinical CV abnormalities. For instance since AD is measured by the degree of vasoconstriction, our data does not necessarily indicate a direct relationship to the abnormal CV response but does provide evidence that vessel responses change following SCI.

The histology and immunohistochemistry analyses from the experiments are limited. It could very well be the case that the descending control from RVLM to sympathetic preganglionic neurons is sufficiently disrupted in the mildly injured animals (12.5g-cm). Although this is highly unlikely, we are limited to interpretations made from densely stained myelin and not other markers for spared axons. Furthermore, there was no quantification of resting circulating levels of catecholamines, which is an important piece of information to acquire especially when conducting exercise experiments. Although we acquired as much data as possible from these animals there is always the feeling that more could have been completed.

Clinical implications and future directions

There has been a significant struggle in the scientific community to generate a relevant model of contusion SCI that mirrors the clinical scenario in regards to CV function. In the clinical situation, an injury decentralizes the ANS and creates a new baseline of cardiac function, which is then further exacerbated by extreme, forced inactivity. Patients are acutely bed ridden and subsequently wheelchair bound leading to chronic cardiac unloading and ultimately cardiac decline. In the pre-clinical scenario, especially in the rodent model, there has been little attention given to the amount of activity rodents are generating spontaneously in their cages. Hence, movement/activity maintains pre-load via muscle pumps and positively influences cardiac health. Our inability to prevent rodents from retraining themselves ultimately results in a model that does not mirror the clinical scenario. From these set of experiments we have found that controlling the spontaneous activity by limiting the amount of space available results in a model more repetitive of what occurs clinically. We demonstrated the CV function is diminished following a T2 25g-cm in standard housing conditions and that it was then exacerbated when we controlled spontaneous activity by limiting the amount of space available to move. Moving forward we can apply exercise interventions to attempt to reverse that cardiac dysfunction. However, it is imperative that any intervention seeking to favorably influence CV function is timed appropriately and is also the right stimulus. We have also found from these sets of experiments that there is not just cardiac systolic dysfunction but that contractility of the heart independent of loading conditions is altered. The exercise interventions utilized here, PHLC and SWIM, neither have the capacity to correct pressure generating although PHLC does reverse systolic dysfunction. It may be

the case moving forward that a combination of interventions are necessary to combat all cardiac abnormalities. There is currently a school of thought that too much damage will be done to persons with SCI if increased levels of activity or exercise interventions are implemented acutely.

Our data further suggests that there are changes in the vasculature that are not fully understood and likely contribute to overall cardiovascular dysfunction. Exercise and/or increased daily physical activity has the potential to help remedy those vascular changes and data generated from the exercise comparison study supports this. It is well established from clinical studies that there is vascular remodeling occurring early post-SCI and implementing an appropriate stimulus could prevent that remodeling. A gap in our current understanding is how increasing activity or implementing an exercise can positively influence *both* the vasculature and the heart. Furthermore, the blood pressure regulating beds have been wildly neglected. These vessels are difficult to study in the clinical setting due to how inaccessible they are. This is where translation research can provide insights to systems that cannot necessarily be studied in humans. Fortunately, with the tiny cage and moderate contusion SCI we are now able to generate a scenario that closely mimics the clinically situation and utilize it as a new baseline to investigate the effects of interventions on the entirety (heart and vasculature) of the CV system.

REFERENCES

Ashley, E. A., J. J. Laskin, L. M. Olenik, R. Burnham, R. D. Steadward, D. C. Cumming and G. D. Wheeler (1993). "Evidence of autonomic dysreflexia during functional electrical stimulation in individuals with spinal cord injuries." Paraplegia **31**(9): 593-605.

Badea, C. T., L. W. Hedlund, J. Cook, B. R. Berridge and G. A. Johnson (2011). "Micro-CT imaging assessment of dobutamine-induced cardiac stress in rats." J Pharmacol Toxicol Methods **63**(1): 24-29.

Ballaz, L., N. Fusco, A. Cretual, B. Langella and R. Brissot (2007). "Acute peripheral blood flow response induced by passive leg cycle exercise in people with spinal cord injury." Arch Phys Med Rehabil **88**(4): 471-476.

Ballaz, L., N. Fusco, A. Cretual, B. Langella and R. Brissot (2008). "Peripheral vascular changes after home-based passive leg cycle exercise training in people with paraplegia: a pilot study." Arch Phys Med Rehabil **89**(11): 2162-2166.

Baselli, G., S. Cerutti, S. Civardi, D. Liberati, F. Lombardi, A. Malliani and M. Pagani (1986). "Spectral and cross-spectral analysis of heart rate and arterial blood pressure variability signals." Comput Biomed Res **19**(6): 520-534.

Basso, D. M., M. S. Beattie and J. C. Bresnahan (1995). "A sensitive and reliable locomotor rating scale for open field testing in rats." J Neurotrauma **12**(1): 1-21.

Bauman, W. A. and A. M. Spungen (2008). "Coronary heart disease in individuals with spinal cord injury: assessment of risk factors." Spinal Cord **46**(7): 466-476.

Bauman, W. A., A. M. Spungen, Y. G. Zhong, J. L. Rothstein, C. Petry and S. K. Gordon (1992). "Depressed serum high density lipoprotein cholesterol levels in veterans with spinal cord injury." Paraplegia **30**(10): 697-703.

Bignami, G. (1996). "Economical test methods for developmental neurobehavioral toxicity." Environ Health Perspect **104 Suppl 2**: 285-298.

Bilello, J. F., J. W. Davis, M. A. Cunningham, T. F. Groom, D. Lemaster and L. P. Sue (2003). "Cervical spinal cord injury and the need for cardiovascular intervention." Arch Surg **138**(10): 1127-1129.

Brock, J. A., M. Yeoh and E. M. McLachlan (2006). "Enhanced neurally evoked responses and inhibition of norepinephrine reuptake in rat mesenteric arteries after spinal transection." Am J Physiol Heart Circ Physiol **290**(1): H398-405.

Burkhoff, D., I. Mirsky and H. Suga (2005). "Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers." Am J Physiol Heart Circ Physiol **289**(2): H501-512.

Cariga, P., M. Catley, C. J. Mathias, G. Savic, H. L. Frankel and P. H. Ellaway (2002). "Organisation of the sympathetic skin response in spinal cord injury." J Neurol Neurosurg Psychiatry **72**(3): 356-360.

Caudle, K. L., D. A. Atkinson, E. H. Brown, K. Donaldson, E. Seibt, T. Chea, E. Smith, K. Chung, A. Shum-Siu, C. C. Cron and D. S. Magnuson (2014). "Hindlimb Stretching Alters Locomotor Function After Spinal Cord Injury in the Adult Rat." Neurorehabil Neural Repair.

Caudle, K. L., E. H. Brown, A. Shum-Siu, D. A. Burke, T. S. Magnuson, M. J. Voor and D. S. Magnuson (2011). "Hindlimb immobilization in a wheelchair alters functional recovery following contusive spinal cord injury in the adult rat." Neurorehabil Neural Repair **25**(8): 729-739.

Cerutti, C., C. Barres and C. Paultre (1994). "Baroreflex modulation of blood pressure and heart rate variabilities in rats: assessment by spectral analysis." Am J Physiol **266**(5 Pt 2): H1993-2000.

Cerutti, C., M. P. Gustin, C. Z. Paultre, M. Lo, C. Julien, M. Vincent and J. Sassard (1991). "Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach." Am J Physiol **261**(4 Pt 2): H1292-1299.

Claydon, V. E., A. T. Hol, J. J. Eng and A. V. Krassioukov (2006). "Cardiovascular responses and postexercise hypotension after arm cycling exercise in subjects with spinal cord injury." Arch Phys Med Rehabil **87**(8): 1106-1114.

Claydon, V. E. and A. V. Krassioukov (2006). "Orthostatic hypotension and autonomic pathways after spinal cord injury." J Neurotrauma **23**(12): 1713-1725.

Collins, H. L. and S. E. Dicarlo (2002). "Acute exercise reduces the response to colon distension in T(5) spinal rats." Am J Physiol Heart Circ Physiol **282**(4): H1566-1570.

Conrad, C. H., W. W. Brooks, J. A. Hayes, S. Sen, K. G. Robinson and O. H. Bing (1995). "Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat." Circulation **91**(1): 161-170.

Contributors, A. Krassioukov, C. F. Biering-Sorensen, W. Donovan, M. Kennelly, S. Kirshblum, K. Krogh, M. S. Alexander, L. Vogel and J. And Wecht (2012). "International Standards to document remaining Autonomic Function after Spinal Cord Injury (ISAFSCI), First Edition 2012." Top Spinal Cord Inj Rehabil **18**(3): 282-296.

Cowan, R. E. and M. S. Nash (2010). "Cardiovascular disease, SCI and exercise: unique risks and focused countermeasures." Disabil Rehabil **32**(26): 2228-2236.

Cragg, J. J., V. K. Noonan, A. Krassioukov and J. Borisoff (2013). "Cardiovascular disease and spinal cord injury: results from a national population health survey." Neurology **81**(8): 723-728.

Currie, K. D., C. R. West, M. Hubli, C. M. Gee and A. V. Krassioukov (2014). "Peak Heart Rates and Sympathetic Function in Tetraplegic Nonathletes and Athletes." Med Sci Sports Exerc.

Currie, K. D., C. R. West and A. V. Krassioukov (2016). "Differences in Left Ventricular Global Function and Mechanics in Paralympic Athletes with Cervical and Thoracic Spinal Cord Injuries." Front Physiol **7**: 110.

Currie, K. D., C. R. West, E. J. Stohr and A. V. Krassioukov (2016). "Left Ventricular Mechanics in Untrained and Trained Males with Tetraplegia." J Neurotrauma.

Curt, A., B. Nitsche, B. Rodic, B. Schurch and V. Dietz (1997). "Assessment of autonomic dysreflexia in patients with spinal cord injury." J Neurol Neurosurg Psychiatry **62**(5): 473-477.

Curt, A., C. Weinhardt and V. Dietz (1996). "Significance of sympathetic skin response in the assessment of autonomic failure in patients with spinal cord injury." J Auton Nerv Syst **61**(2): 175-180.

Cybulski, G. R., R. D. Penn and R. J. Jaeger (1984). "Lower extremity functional neuromuscular stimulation in cases of spinal cord injury." Neurosurgery **15**(1): 132-146.

Dampney, R. A. (1994). "Functional organization of central pathways regulating the cardiovascular system." Physiol Rev **74**(2): 323-364.

Davis, G. M., R. J. Shephard and F. H. Leenen (1987). "Cardiac effects of short term arm crank training in paraplegics: echocardiographic evidence." Eur J Appl Physiol Occup Physiol **56**(1): 90-96.

de Groot, P. C., M. W. Bleeker, D. H. van Kuppevelt, L. H. van der Woude and M. T. Hopman (2006). "Rapid and extensive arterial adaptations after spinal cord injury." Arch Phys Med Rehabil **87**(5): 688-696.

De Groot, P. C., D. H. Van Kuppevelt, C. Pons, G. Snoek, L. H. Van Der Woude and M. T. Hopman (2003). "Time course of arterial vascular adaptations to inactivity and paralyses in humans." Med Sci Sports Exerc **35**(12): 1977-1985.

Despres, J. P., S. Moorjani, P. J. Lupien, A. Tremblay, A. Nadeau and C. Bouchard (1990). "Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease." Arteriosclerosis **10**(4): 497-511.

DeVeau, K. M., E. K. Martin, N. T. King, A. Shum-Siu, B. B. Keller, C. R. West and D. S. Magnuson (2016). "Challenging cardiac function post-spinal cord injury with dobutamine." Auton Neurosci.

Devillard, X., D. Rimaud, F. Roche and P. Calmels (2007). "Effects of training programs for spinal cord injury." Ann Readapt Med Phys **50**(6): 490-498, 480-499.

Ditor, D. S., M. J. Macdonald, M. V. Kamath, J. Bugaresti, M. Adams, N. McCartney and A. L. Hicks (2005). "The effects of body-weight supported treadmill training on cardiovascular regulation in individuals with motor-complete SCI." Spinal Cord **43**(11): 664-673.

Ditunno, J. F., J. W. Little, A. Tessler and A. S. Burns (2004). "Spinal shock revisited: a four-phase model." Spinal Cord **42**(7): 383-395.

Dixit, S. (1995). "Bradycardia associated with high cervical spinal cord injury." Surg Neurol **43**(5): 514.

Dolinak, D. and E. Balraj (2007). "Autonomic dysreflexia and sudden death in people with traumatic spinal cord injury." Am J Forensic Med Pathol **28**(2): 95-98.

Edwards, C. H., 2nd, J. S. Rankin, P. A. McHale, D. Ling and R. W. Anderson (1981). "Effects of ischemia on left ventricular regional function in the conscious dog." Am J Physiol **240**(3): H413-420.

Eltorai, I., R. Kim, M. Vulpe, H. Kasravi and W. Ho (1992). "Fatal cerebral hemorrhage due to autonomic dysreflexia in a tetraplegic patient: case report and review." Paraplegia **30**(5): 355-360.

Espinosa-Medina, I., O. Saha, F. Boismoreau, Z. Chettouh, F. Rossi, W. D. Richardson and J. F. Brunet (2016). "The sacral autonomic outflow is sympathetic." Science **354**(6314): 893-897.

Eysmann, S. B., P. S. Douglas, S. E. Katz, M. Sarkarati and J. Y. Wei (1995). "Left ventricular mass and diastolic filling patterns in quadriplegia and implications for effects of normal aging on the heart." Am J Cardiol **75**(2): 201-203.

Faghri, P. D. and J. Yount (2002). "Electrically induced and voluntary activation of physiologic muscle pump: a comparison between spinal cord-injured and able-bodied individuals." Clin Rehabil **16**(8): 878-885.

Frisbie, J. H. and D. J. Steele (1997). "Postural hypotension and abnormalities of salt and water metabolism in myelopathy patients." Spinal Cord **35**(5): 303-307.

Furlan, J. C. and M. G. Fehlings (2008). "Cardiovascular complications after acute spinal cord injury: pathophysiology, diagnosis, and management." Neurosurg Focus **25**(5): E13.

Furlan, J. C., M. G. Fehlings, P. Shannon, M. D. Norenberg and A. V. Krassioukov (2003). "Descending vasomotor pathways in humans: correlation between axonal preservation and cardiovascular dysfunction after spinal cord injury." J Neurotrauma **20**(12): 1351-1363.

Garshick, E., A. Kelley, S. A. Cohen, A. Garrison, C. G. Tun, D. Gagnon and R. Brown (2005). "A prospective assessment of mortality in chronic spinal cord injury." Spinal Cord **43**(7): 408-416.

Gater, D. R., Jr. (2007). "Obesity after spinal cord injury." Phys Med Rehabil Clin N Am **18**(2): 333-351, vii.

Gates, P. E., I. G. Campbell and K. P. George (2002). "Absence of training-specific cardiac adaptation in paraplegic athletes." Med Sci Sports Exerc **34**(11): 1699-1704.

Gilroy, A. M. and B. R. MacPherson (2106). Atlas of Anatomy. New York, Thieme.

Ginis, K. A., A. L. Hicks, A. E. Latimer, D. E. Warburton, C. Bourne, D. S. Ditor, D. L. Goodwin, K. C. Hayes, N. McCartney, A. McIlraith, P. Pomerleau, K. Smith, J. A. Stone and D. L. Wolfe (2011). "The development of evidence-informed physical activity guidelines for adults with spinal cord injury." Spinal Cord **49**(11): 1088-1096.

Gonzalez, F., J. Y. Chang, K. Banovac, D. Messina, A. Martinez-Arizala and R. E. Kelley (1991). "Autoregulation of cerebral blood flow in patients with orthostatic hypotension after spinal cord injury." Paraplegia **29**(1): 1-7.

Griffin, L., M. J. Decker, J. Y. Hwang, B. Wang, K. Kitchen, Z. Ding and J. L. Ivy (2009). "Functional electrical stimulation cycling improves body composition, metabolic and neural factors in persons with spinal cord injury." J Electromyogr Kinesiol **19**(4): 614-622.

Grimm, D. R., R. E. De Meersman, P. L. Almenoff, A. M. Spungen and W. A. Bauman (1997). "Sympathovagal balance of the heart in subjects with spinal cord injury." Am J Physiol **272**(2 Pt 2): H835-842.

Guly, H. R., O. Bouamra, F. E. Lecky, A. Trauma and N. Research (2008). "The incidence of neurogenic shock in patients with isolated spinal cord injury in the emergency department." Resuscitation **76**(1): 57-62.

Gupta, N., K. T. White and P. R. Sandford (2006). "Body mass index in spinal cord injury -- a retrospective study." Spinal Cord **44**(2): 92-94.

Hadi, B., Y. P. Zhang, D. A. Burke, C. B. Shields and D. S. Magnuson (2000). "Lasting paraplegia caused by loss of lumbar spinal cord interneurons in rats: no direct correlation with motor neuron loss." J Neurosurg **93**(2 Suppl): 266-275.

Hector, S. M., T. Biering-Sorensen, A. Krassioukov and F. Biering-Sorensen (2013). "Cardiac arrhythmias associated with spinal cord injury." J Spinal Cord Med **36**(6): 591-599.

Helkowski, W. M., J. F. Ditunno, Jr. and M. Boninger (2003). "Autonomic dysreflexia: incidence in persons with neurologically complete and incomplete tetraplegia." J Spinal Cord Med **26**(3): 244-247.

Ho, C. P. and A. V. Krassioukov (2010). "Autonomic dysreflexia and myocardial ischemia." Spinal Cord **48**(9): 714-715.

Hopman, M. T., C. Dueck, M. Monroe, W. T. Philips and J. S. Skinner (1998). "Limits to maximal performance in individuals with spinal cord injury." Int J Sports Med **19**(2): 98-103.

Hopman, M. T., B. Oeseburg and R. A. Binkhorst (1992). "Cardiovascular responses in paraplegic subjects during arm exercise." Eur J Appl Physiol Occup Physiol **65**(1): 73-78.

Houle, J. D., K. Morris, R. D. Skinner, E. Garcia-Rill and C. A. Peterson (1999). "Effects of fetal spinal cord tissue transplants and cycling exercise on the soleus muscle in spinalized rats." Muscle Nerve **22**(7): 846-856.

Houtman, S., B. Oeseburg and M. T. Hopman (2000). "Blood volume and hemoglobin after spinal cord injury." Am J Phys Med Rehabil **79**(3): 260-265.

Hubli, M., C. M. Gee and A. V. Krassioukov (2015). "Refined assessment of blood pressure instability after spinal cord injury." Am J Hypertens **28**(2): 173-181.

Huonker, M., A. Schmid, S. Sorichter, A. Schmidt-Trucksab, P. Mrosek and J. Keul (1998). "Cardiovascular differences between sedentary and wheelchair-trained subjects with paraplegia." Med Sci Sports Exerc **30**(4): 609-613.

Hutchinson, K. J., F. Gomez-Pinilla, M. J. Crowe, Z. Ying and D. M. Basso (2004). "Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats." Brain **127**(Pt 6): 1403-1414.

Illman, A., K. Stiller and M. Williams (2000). "The prevalence of orthostatic hypotension during physiotherapy treatment in patients with an acute spinal cord injury." Spinal Cord **38**(12): 741-747.

Inskip, J. A., L. M. Ramer, M. S. Ramer, A. V. Krassioukov and V. E. Claydon (2012). "Spectral analyses of cardiovascular control in rodents with spinal cord injury." J Neurotrauma **29**(8): 1638-1649.

Kalincik, T., E. A. Choi, F. Feron, J. Bianco, R. Sutharsan, I. Hayward, A. Mackay-Sim, P. Carrive and P. M. Waite (2010). "Olfactory ensheathing cells reduce duration of autonomic dysreflexia in rats with high spinal cord injury." Auton Neurosci **154**(1-2): 20-29.

Kass, D. A. and R. P. Kelly (1992). "Ventriculo-arterial coupling: concepts, assumptions, and applications." Ann Biomed Eng **20**(1): 41-62.

Kass, D. A., W. L. Maughan, Z. M. Guo, A. Kono, K. Sunagawa and K. Sagawa (1987). "Comparative influence of load versus inotropic states on indexes of ventricular contractility: experimental and theoretical analysis based on pressure-volume relationships." Circulation **76**(6): 1422-1436.

Kass, D. A., T. Yamazaki, D. Burkhoff, W. L. Maughan and K. Sagawa (1986). "Determination of left ventricular end-systolic pressure-volume relationships by the conductance (volume) catheter technique." Circulation **73**(3): 586-595.

Kessler, K. M., I. Pina, B. Green, B. Burnett, M. Laighold, M. Bilsker, A. R. Palomo and R. J. Myerburg (1986). "Cardiovascular findings in quadriplegic and paraplegic patients and in normal subjects." Am J Cardiol **58**(6): 525-530.

Kirshblum, S. C., F. Biering-Sorensen, R. Betz, S. Burns, W. Donovan, D. E. Graves, M. Johansen, L. Jones, M. J. Mulcahey, G. M. Rodriguez, M. Schmidt-Read, J. D. Steeves, K. Tansey and W. Waring (2014). "International standards for neurological classification of spinal cord injury: cases with classification challenges." Top Spinal Cord Inj Rehabil **20**(2): 81-89.

Krahwinkel, W., T. Ketteler, J. Godke, J. Wolfertz, L. J. Ulbricht, I. Krakau and H. Gulker (1997). "Dobutamine stress echocardiography." Eur Heart J **18 Suppl D**: D9-15.

Krassioukov, A. (2009). "Autonomic function following cervical spinal cord injury." Respir Physiol Neurobiol **169**(2): 157-164.

Krassioukov, A. and V. E. Claydon (2006). "The clinical problems in cardiovascular control following spinal cord injury: an overview." Prog Brain Res **152**: 223-229.

Krassioukov, A., D. E. Warburton, R. Teasell, J. J. Eng and T. Spinal Cord Injury Rehabilitation Evidence Research (2009). "A systematic review of the management of autonomic dysreflexia after spinal cord injury." Arch Phys Med Rehabil **90**(4): 682-695.

Krassioukov, A. V., J. C. Furlan and M. G. Fehlings (2003). "Autonomic dysreflexia in acute spinal cord injury: an under-recognized clinical entity." J Neurotrauma **20**(8): 707-716.

Krassioukov, A. V. and S. J. Harkema (2006). "Effect of harness application and postural changes on cardiovascular parameters of individuals with spinal cord injury." Spinal Cord **44**(12): 780-786.

Krenz, N. R., S. O. Meakin, A. V. Krassioukov and L. C. Weaver (1999). "Neutralizing intraspinal nerve growth factor blocks autonomic dysreflexia caused by spinal cord injury." J Neurosci **19**(17): 7405-7414.

Krenz, N. R. and L. C. Weaver (1998). "Changes in the morphology of sympathetic preganglionic neurons parallel the development of autonomic dysreflexia after spinal cord injury in rats." Neurosci Lett **243**(1-3): 61-64.

Krenz, N. R. and L. C. Weaver (1998). "Sprouting of primary afferent fibers after spinal cord transection in the rat." Neuroscience **85**(2): 443-458.

Krum, H., W. J. Louis, D. J. Brown and L. G. Howes (1992). "Pressor dose responses and baroreflex sensitivity in quadriplegic spinal cord injury patients." J Hypertens **10**(3): 245-250.

Kuerzi, J., E. H. Brown, A. Shum-Siu, A. Siu, D. Burke, J. Morehouse, R. R. Smith and D. S. Magnuson (2010). "Task-specificity vs. ceiling effect: step-training in shallow water after spinal cord injury." Exp Neurol **224**(1): 178-187.

Laird, A. S., P. Carrive and P. M. Waite (2006). "Cardiovascular and temperature changes in spinal cord injured rats at rest and during autonomic dysreflexia." J Physiol **577**(Pt 2): 539-548.

Laird, A. S., P. Carrive and P. M. Waite (2009). "Effect of treadmill training on autonomic dysreflexia in spinal cord--injured rats." Neurorehabil Neural Repair **23**(9): 910-920.

Laird, A. S., A. M. Finch, P. M. Waite and P. Carrive (2008). "Peripheral changes above and below injury level lead to prolonged vascular responses following high spinal cord injury." Am J Physiol Heart Circ Physiol **294**(2): H785-792.

Lehmann, K. G., J. G. Lane, J. M. Piepmeier and W. P. Batsford (1987). "Cardiovascular abnormalities accompanying acute spinal cord injury in humans: incidence, time course and severity." J Am Coll Cardiol **10**(1): 46-52.

Leite, S., J. Oliveira-Pinto, M. Tavares-Silva, M. Abdellatif, D. Fontoura, I. Falcao-Pires, A. F. Leite-Moreira and A. P. Lourenco (2015). "Echocardiography and invasive hemodynamics during stress testing for diagnosis of heart failure with preserved ejection fraction: an experimental study." Am J Physiol Heart Circ Physiol **308**(12): H1556-1563.

Levine, B. D., J. H. Zuckerman and J. A. Pawelczyk (1997). "Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance." Circulation **96**(2): 517-525.

Lualdi, J. C. and P. S. Douglas (1997). "Echocardiography for the assessment of myocardial viability." J Am Soc Echocardiogr **10**(7): 772-780.

Lujan, H. L. and S. E. DiCarlo (2007). "T5 spinal cord transection increases susceptibility to reperfusion-induced ventricular tachycardia by enhancing sympathetic activity in conscious rats." Am J Physiol Heart Circ Physiol **293**(6): H3333-3339.

Lujan, H. L. and S. E. Dicarlo (2014). "Increasing venous return as a strategy to prevent or reverse cardiac dysfunction following spinal cord injury." J Physiol **592**(Pt 8): 1727-1728.

Lujan, H. L., H. Janbaih and S. E. DiCarlo (2012). "Dynamic interaction between the heart and its sympathetic innervation following T5 spinal cord transection." J Appl Physiol (1985) **113**(8): 1332-1341.

Lujan, H. L., H. Janbaih and S. E. DiCarlo (2014). "Structural remodeling of the heart and its premotor cardioinhibitory vagal neurons following T(5) spinal cord transection." J Appl Physiol (1985) **116**(9): 1148-1155.

Lujan, H. L., H. Janbaih, H. Z. Feng, J. P. Jin and S. E. DiCarlo (2012). "Ventricular function during exercise in mice and rats." Am J Physiol Regul Integr Comp Physiol **302**(1): R68-74.

Lujan, H. L., G. Palani and S. E. DiCarlo (2010). "Structural neuroplasticity following T5 spinal cord transection: increased cardiac sympathetic innervation density and SPN arborization." Am J Physiol Regul Integr Comp Physiol **299**(4): R985-995.

Machhada, A., N. Marina, A. Korsak, D. J. Stuckey, M. F. Lythgoe and A. V. Gourine (2016). "Origins of the vagal drive controlling left ventricular contractility." J Physiol **594**(14): 4017-4030.

Magnuson, D. S., R. Lovett, C. Coffee, R. Gray, Y. Han, Y. P. Zhang and D. A. Burke (2005). "Functional consequences of lumbar spinal cord contusion injuries in the adult rat." J Neurotrauma **22**(5): 529-543.

Magnuson, D. S., R. R. Smith, E. H. Brown, G. Enzmann, C. Angeli, P. M. Quesada and D. Burke (2009). "Swimming as a model of task-specific locomotor retraining after spinal cord injury in the rat." Neurorehabil Neural Repair **23**(6): 535-545.

Magnuson, D. S., T. C. Trinder, Y. P. Zhang, D. Burke, D. J. Morassutti and C. B. Shields (1999). "Comparing deficits following excitotoxic and contusion injuries in the thoracic and lumbar spinal cord of the adult rat." Exp Neurol **156**(1): 191-204.

Mallek, J. T., K. Inaba, B. C. Branco, C. Ives, L. Lam, P. Talving, J. S. David and D. Demetriades (2012). "The incidence of neurogenic shock after spinal cord injury in patients admitted to a high-volume level I trauma center." Am Surg **78**(5): 623-626.

Mathias, C. J. (1995). "Orthostatic hypotension: causes, mechanisms, and influencing factors." Neurology **45**(4 Suppl 5): S6-11.

Mathias, C. J., N. J. Christensen, J. L. Corbett, H. L. Frankel, T. J. Goodwin and W. S. Peart (1975). "Plasma catecholamines, plasma renin activity and plasma aldosterone in tetraplegic man, horizontal and tilted." Clin Sci Mol Med **49**(4): 291-299.

Mathias, C. J., N. J. Christensen, H. L. Frankel and W. S. Peart (1980). "Renin release during head-up tilt occurs independently of sympathetic nervous activity in tetraplegic man." Clin Sci (Lond) **59**(4): 251-256.

Mathias, C. J., H. L. Frankel, N. J. Christensen and J. M. Spalding (1976). "Enhanced pressor response to noradrenaline in patients with cervical spinal cord transection." Brain **99**(4): 757-770.

Mathias, C. J., R. Mallipeddi and K. Bleasdale-Barr (1999). "Symptoms associated with orthostatic hypotension in pure autonomic failure and multiple system atrophy." J Neurol **246**(10): 893-898.

Mayorov, D. N., M. A. Adams and A. V. Krassioukov (2001). "Telemetric blood pressure monitoring in conscious rats before and after compression injury of spinal cord." J Neurotrauma **18**(7): 727-736.

Nash, M. S., S. Bilsker, A. E. Marcillo, S. M. Isaac, L. A. Botelho, K. J. Klose, B. A. Green, M. T. Rountree and J. D. Shea (1991). "Reversal of adaptive left ventricular atrophy following electrically-stimulated exercise training in human tetraplegics." Paraplegia **29**(9): 590-599.

Nash, M. S. and P. L. Jacobs (1998). "Cardiac structure and function in exercise trained and sedentary persons with paraplegia." Med Sci Sports Exerc **30**(8): 1336-1338.

Nout, Y. S., M. S. Beattie and J. C. Bresnahan (2012). "Severity of locomotor and cardiovascular derangements after experimental high-thoracic spinal cord injury is anesthesia dependent in rats." J Neurotrauma **29**(5): 990-999.

Pacher, P., T. Nagayama, P. Mukhopadhyay, S. Batkai and D. A. Kass (2008). "Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats." Nat Protoc **3**(9): 1422-1434.

Pan, S. L., Y. H. Wang, H. L. Lin, C. W. Chang, T. Y. Wu and E. T. Hsieh (2005). "Intracerebral hemorrhage secondary to autonomic dysreflexia in a young person with incomplete C8 tetraplegia: A case report." Arch Phys Med Rehabil **86**(3): 591-593.

Pellikka, P. A., V. L. Roger, J. K. Oh, F. A. Miller, J. B. Seward and A. J. Tajik (1995). "Stress echocardiography. Part II. Dobutamine stress echocardiography: techniques, implementation, clinical applications, and correlations." Mayo Clin Proc **70**(1): 16-27.

Perhonen, M. A., F. Franco, L. D. Lane, J. C. Buckey, C. G. Blomqvist, J. E. Zerwekh, R. M. Peshock, P. T. Weatherall and B. D. Levine (2001). "Cardiac atrophy after bed rest and spaceflight." J Appl Physiol (1985) **91**(2): 645-653.

Perhonen, M. A., J. H. Zuckerman and B. D. Levine (2001). "Deterioration of left ventricular chamber performance after bed rest : "cardiovascular deconditioning" or hypovolemia?" Circulation **103**(14): 1851-1857.

Pierard, L. A., C. Berthe, A. Albert, J. Carlier and H. E. Kulbertus (1989). "Haemodynamic alterations during ischaemia induced by dobutamine stress testing." Eur Heart J **10**(9): 783-790.

Pine, Z. M., S. D. Miller and J. A. Alonso (1991). "Atrial fibrillation associated with autonomic dysreflexia." Am J Phys Med Rehabil **70**(5): 271-273.

Plante, E., D. Lachance, M. C. Drolet, E. Roussel, J. Couet and M. Arsenault (2005). "Dobutamine stress echocardiography in healthy adult male rats." Cardiovasc Ultrasound **3**: 34.

Popok, D. W., C. R. West, L. McCracken and A. V. Krassioukov (2016). "Effects of early and delayed initiation of exercise training on cardiac and hemodynamic function post spinal cord injury." Exp Physiol.

Puzserova, A., V. Ilovska, P. Balis, P. Slezak and I. Bernatova (2014). "Age-related alterations in endothelial function of femoral artery in young SHR and WKY rats." Biomed Res Int **2014**: 658479.

Radovits, T., A. Olah, A. Lux, B. T. Nemeth, L. Hidi, E. Birtalan, D. Kellermayer, C. Matyas, G. Szabo and B. Merkely (2013). "Rat model of exercise-induced cardiac hypertrophy: hemodynamic characterization using left ventricular pressure-volume analysis." Am J Physiol Heart Circ Physiol **305**(1): H124-134.

Ravensbergen, H. J., M. L. Walsh, A. V. Krassioukov and V. E. Claydon (2012). "Electrocardiogram-based predictors for arrhythmia after spinal cord injury." Clin Auton Res **22**(6): 265-273.

Raymond, J., G. M. Davis, G. Bryant and J. Clarke (1999). "Cardiovascular responses to an orthostatic challenge and electrical-stimulation-induced leg muscle contractions in individuals with paraplegia." Eur J Appl Physiol Occup Physiol **80**(3): 205-212.

Reis, D. J., S. Morrison and D. A. Ruggiero (1988). "The C1 area of the brainstem in tonic and reflex control of blood pressure. State of the art lecture." Hypertension **11**(2 Pt 2): I8-13.

Rosado-Rivera, D., M. Radulovic, J. P. Handrakis, C. M. Cirnigliaro, A. M. Jensen, S. Kirshblum, W. A. Bauman and J. M. Wecht (2011). "Comparison of 24-hour cardiovascular and autonomic function in paraplegia, tetraplegia, and control groups: implications for cardiovascular risk." J Spinal Cord Med **34**(4): 395-403.

Round, A. M., S. E. Park, K. Walden, V. K. Noonan, A. F. Townson and A. V. Krassioukov (2016). "An evaluation of the International Standards to Document Remaining Autonomic Function after Spinal Cord Injury: input from the international community." Spinal Cord.

Ruffolo, R. R., Jr. (1987). "The pharmacology of dobutamine." Am J Med Sci **294**(4): 244-248.

Ruggiero, D. A., S. L. Cravo, V. Arango and D. J. Reis (1989). "Central control of the circulation by the rostral ventrolateral reticular nucleus: anatomical substrates." Prog Brain Res **81**: 49-79.

Rummery, N. M., D. Tripovic, E. M. McLachlan and J. A. Brock (2010). "Sympathetic vasoconstriction is potentiated in arteries caudal but not rostral to a spinal cord transection in rats." J Neurotrauma **27**(11): 2077-2089.

Sahota, I. S., H. R. Ravensbergen, M. S. McGrath and V. E. Claydon (2012). "Cerebrovascular responses to orthostatic stress after spinal cord injury." J Neurotrauma **29**(15): 2446-2456.

Sandrow-Feinberg, H. R., J. Izzi, J. S. Shumsky, V. Zhukareva and J. D. Houle (2009). "Forced exercise as a rehabilitation strategy after unilateral cervical spinal cord contusion injury." J Neurotrauma **26**(5): 721-731.

Schmid, A., M. Halle, C. Stutzle, D. Konig, M. W. Baumstark, M. J. Storch, A. Schmidt-Trucksass, M. Lehmann, A. Berg and J. Keul (2000). "Lipoproteins and free plasma catecholamines in spinal cord injured men with different injury levels." Clin Physiol **20**(4): 304-310.

Schmid, A., M. Huonker, J. M. Barturen, F. Stahl, A. Schmidt-Trucksass, D. Konig, D. Grathwohl, M. Lehmann and J. Keul (1998). "Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury." J Appl Physiol (1985) **85**(2): 635-641.

Schneider, C., K. Jaquet, S. Geidel, R. Malisius, S. Boczor, T. Rau, T. Zienkiewicz, D. Hennig, K. H. Kuck and K. Krause (2010). "Regional diastolic and systolic function by strain rate imaging for the detection of intramural viability during dobutamine stress echocardiography in a porcine model of myocardial infarction." Echocardiography **27**(5): 552-562.

Schucht, P., O. Raineteau, M. E. Schwab and K. Fouad (2002). "Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord." Exp Neurol **176**(1): 143-153.

Sclater, A. and K. Alagiakrishnan (2004). "Orthostatic hypotension. A primary care primer for assessment and treatment." Geriatrics **59**(8): 22-27.

Segreti, J. A., K. C. Marsh, J. S. Polakowski and R. M. Fryer (2008). "Evoked changes in cardiovascular function in rats by infusion of levosimendan, OR-1896 [(R)-N-(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)acetamide], OR-1855 [(R)-6-(4-aminophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one], dobutamine, and milrinone: comparative effects on peripheral resistance, cardiac output, dP/dt, pulse rate, and blood pressure." J Pharmacol Exp Ther **325**(1): 331-340.

Silver, J. R. (2000). "Early autonomic dysreflexia." Spinal Cord **38**(4): 229-233.

Smith, R. R., D. A. Burke, A. D. Baldini, A. Shum-Siu, R. Baltzley, M. Bunger and D. S. Magnuson (2006). "The Louisville Swim Scale: a novel assessment of hindlimb function following spinal cord injury in adult rats." J Neurotrauma **23**(11): 1654-1670.

Smith, R. R., A. Shum-Siu, R. Baltzley, M. Bunger, A. Baldini, D. A. Burke and D. S. Magnuson (2006). "Effects of swimming on functional recovery after incomplete spinal cord injury in rats." J Neurotrauma **23**(6): 908-919.

Squair, J. W., C. R. West, D. Popok, P. Assinck, J. Liu, W. Tetzlaff and A. V. Krassioukov (2016). "High Thoracic Contusion Model for the Investigation of Cardiovascular Function after Spinal Cord Injury." J Neurotrauma.

Strack, A. M., W. B. Sawyer, L. M. Marubio and A. D. Loewy (1988). "Spinal origin of sympathetic preganglionic neurons in the rat." Brain Res **455**(1): 187-191.

Sugawara, J., K. Hayashi, F. Kaneko, H. Yamada, T. Kizuka and H. Tanaka (2004). "Reductions in basal limb blood flow and lumen diameter after short-term leg casting." Med Sci Sports Exerc **36**(10): 1689-1694.

Summers, R. L., S. D. Baker, S. A. Sterling, J. M. Porter and A. E. Jones (2013). "Characterization of the spectrum of hemodynamic profiles in trauma patients with acute neurogenic shock." J Crit Care **28**(4): 531 e531-535.

Sunagawa, K., W. L. Maughan, D. Burkhoff and K. Sagawa (1983). "Left ventricular interaction with arterial load studied in isolated canine ventricle." Am J Physiol **245**(5 Pt 1): H773-780.

Svensson, M., H. Siosteen, H. Wetterqvist and L. Sullivan (1995). " Influence of physiotherapy on leg blood flow in patients with complete spinal cord injury lesions. ." Physiother Theory Pract **11**: 97–107.

Teasell, R. W., J. M. Arnold, A. Krassioukov and G. A. Delaney (2000). "Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury." Arch Phys Med Rehabil **81**(4): 506-516.

Thijssen, D. H., P. C. De Groot, A. van den Bogerd, M. Veltmeijer, N. T. Cable, D. J. Green and M. T. Hopman (2012). "Time course of arterial remodelling in diameter and wall thickness above and below the lesion after a spinal cord injury." Eur J Appl Physiol **112**(12): 4103-4109.

Thijssen, D. H., P. Heesterbeek, D. J. van Kuppevelt, J. Duysens and M. T. Hopman (2005). "Local vascular adaptations after hybrid training in spinal cord-injured subjects." Med Sci Sports Exerc **37**(7): 1112-1118.

Valles, M., J. Benito, E. Portell and J. Vidal (2005). "Cerebral hemorrhage due to autonomic dysreflexia in a spinal cord injury patient." Spinal Cord **43**(12): 738-740.

Washburn, R. A., D. D. Savage, S. R. Dearwater, R. E. LaPorte, S. J. Anderson, G. Brenes, L. L. Adams, H. K. Lee, J. Holland, M. Cowan and et al. (1986). "Echocardiographic left ventricular mass and physical activity: quantification of the relation in spinal cord injured and apparently healthy active men." Am J Cardiol **58**(13): 1248-1253.

Wecht, J. M., R. E. de Meersman, J. P. Weir, W. A. Bauman and D. R. Grimm (2000). "Effects of autonomic disruption and inactivity on venous vascular function." Am J Physiol Heart Circ Physiol **278**(2): H515-520.

Wecht, J. M., J. P. Weir, M. Galea, S. Martinez and W. A. Bauman (2015). "Prevalence of abnormal systemic hemodynamics in veterans with and without spinal cord injury." Arch Phys Med Rehabil **96**(6): 1071-1079.

Wecht, J. M., C. Zhu, J. P. Weir, C. Yen, C. Renzi and M. Galea (2013). "A prospective report on the prevalence of heart rate and blood pressure abnormalities in veterans with spinal cord injuries." J Spinal Cord Med **36**(5): 454-462.

West, C. R., A. Alyahya, I. Laher and A. Krassioukov (2013). "Peripheral vascular function in spinal cord injury: a systematic review." Spinal Cord **51**(1): 10-19.

West, C. R., A. Bellantoni and A. V. Krassioukov (2013). "Cardiovascular function in individuals with incomplete spinal cord injury: a systematic review." Top Spinal Cord Inj Rehabil **19**(4): 267-278.

West, C. R., I. G. Campbell, R. E. Shave and L. M. Romer (2012). "Effects of abdominal binding on cardiorespiratory function in cervical spinal cord injury." Respir Physiol Neurobiol **180**(2-3): 275-282.

West, C. R., I. G. Campbell, R. E. Shave and L. M. Romer (2012). "Resting cardiopulmonary function in Paralympic athletes with cervical spinal cord injury." Med Sci Sports Exerc **44**(2): 323-329.

West, C. R., M. A. Crawford, I. Laher, M. S. Ramer and A. V. Krassioukov (2016). "Passive Hind-Limb Cycling Reduces the Severity of Autonomic Dysreflexia After Experimental Spinal Cord Injury." Neurorehabil Neural Repair **30**(4): 317-327.

West, C. R., M. A. Crawford, M. S. Poormasjedi-Meibod, K. D. Currie, A. Fallavollita, V. Yuen, J. H. McNeill and A. V. Krassioukov (2014). "Passive hind-limb cycling improves cardiac function and reduces cardiovascular disease risk in experimental spinal cord injury." J Physiol **592**(Pt 8): 1771-1783.

West, C. R., K. D. Currie, C. Gee, A. V. Krassioukov and J. Borisoff (2015). "Active-Arm Passive-Leg Exercise Improves Cardiovascular Function in Spinal Cord Injury." Am J Phys Med Rehabil **94**(11): e102-106.

West, C. R., C. M. Gee, C. Voss, M. Hubli, K. D. Currie, J. Schmid and A. V. Krassioukov (2015). "Cardiovascular control, autonomic function, and elite endurance performance in spinal cord injury." Scand J Med Sci Sports **25**(4): 476-485.

West, C. R., P. Mills and A. V. Krassioukov (2012). "Influence of the neurological level of spinal cord injury on cardiovascular outcomes in humans: a meta-analysis." Spinal Cord **50**(7): 484-492.

West, C. R., D. Popok, M. A. Crawford and A. V. Krassioukov (2015). "Characterizing the temporal development of cardiovascular dysfunction in response to spinal cord injury." J Neurotrauma **32**(12): 922-930.

West, C. R., L. M. Romer and A. Krassioukov (2013). "Autonomic function and exercise performance in elite athletes with cervical spinal cord injury." Med Sci Sports Exerc **45**(2): 261-267.

West, C. R., J. W. Squair, L. McCracken, K. D. Currie, R. Somvanshi, V. Yuen, A. A. Phillips, U. Kumar, J. H. McNeill and A. V. Krassioukov (2016). "Cardiac Consequences of Autonomic Dysreflexia in Spinal Cord Injury." Hypertension **68**(5): 1281-1289.

Wu, W. C., L. A. Ireland and A. Sadaniantz (2004). "Evaluation of aortic valve disorders using stress echocardiography." Echocardiography **21**(5): 459-466.

Yeoh, M., E. M. McLachlan and J. A. Brock (2004). "Tail arteries from chronically spinalized rats have potentiated responses to nerve stimulation in vitro." J Physiol **556**(Pt 2): 545-555.

Yokota, T., T. Matsunaga, R. Okiyama, K. Hirose, H. Tanabe, T. Furukawa and H. Tsukagoshi (1991). "Sympathetic skin response in patients with multiple sclerosis compared with patients with spinal cord transection and normal controls." Brain **114 (Pt 3)**: 1381-1394.

Zhu, C., M. Galea, E. Livote, D. Signor and J. M. Wecht (2013). "A retrospective chart review of heart rate and blood pressure abnormalities in veterans with spinal cord injury." J Spinal Cord Med **36**(5): 463-475.

LIST OF ABBREVIATIONS

A	peak transmitral filling velocity during late diastole
ACh	acetylcholine
AD	Autonomic dysreflexia
ANOVA	one-way analysis of variance
ANS	autonomic nervous system
ASIA	American Spinal Injury Association
B-mode	Brightness mode
BBB	Basso, Beattie
BP	blood pressure
BPM	beats per minute;
BWSTT	Body weight supported treadmill training
C	cervical
CGRP	calcitonin gene related peptide
CI	cardiac index;
CNS	central nervous system
CNX	Vagus nerve
CO	cardiac output;
CON	control
CV	cardiovascular
CVD	cardiovascular disease
DPB	diastolic blood pressure;
DOB	Dobutamine
DP/dtmax	the maximal slope of LV systolic pressure increment
dP/dtmax-EDV	the slope of the dP/d*t*max end-diastolic volume relationship
DP/dtmin	diastolic pressure decrement
DSE	Dobutamine stress echocardiography
E	transmitral filling velocities during early diastole;
Ea	arterial elastance;
EC	Eriochrome Cyanin
EDPVR	end diastolic pressure volume relationship
EDV	end-diastolic volume;
Ees	end systolic pressure volume relationship;
EF	ejection fraction;
ESV	end-systolic volume;
EtOH	ethanol

FES	Functional electrical stimulation
HR	heart rate;
HT	high thoracic
IML	intermediolateral cell column
ISAFSCI	International Standards to Document remaining Autonomic Function
IVC	inferior vena cava
LC	large cages
LFSBP	low frequency systolic blood pressure;
LT	low thoracic
LV	left ventricle
LVIDd	left ventricular internal diameter during diastole;
LVIDs	left ventricular internal diameter during systole;
LVPWd	left ventricular posterior wall thickness during diastole;
M-mode	motion mode
MAP	mean arterial pressure;
MILD	mild (12.5g-cm)
MOD	moderate/severe (25g-cm)
OH	orthostatic hypotension
ONA	overnight activity
OTC	optimal cutting temperature
PA	physical activity
PBS	phosphate buffered saline
Ped	end diastolic pressure;
Pes	end systolic pressure;
PFA	paraformaldehyde
PHLC	passive hindlimb cycling
PNS	parasympathetic system
PRSW	preload recruitable stroke work;
PSLAX	parasternal long axis
PSS	physiological saline solution
PV	pressure volume
PWT	posterior wall thickness
RM ANOVA	repeated measures analyses of variance
RVLM	rostral ventral lateral medulla
RWT	relative wall thickness;
SAX	parasternal short axis
SBP	systolic blood pressure
SC	spinal cord
SCI	spinal cord injury
SD	Sprague Dawley
SD	standard deviation
Ser	serotonin

SNP	sodium-nitroprusside
SNS	sympathetic nervous system
SSR	sympathetic skin response
SV	stroke volume;
SVR	systemic vascular resistance;
SW	stroke work;
SWI	stroke work index;
SWIM	swim training
T1	thoracic level one
T2	thoracic level two
T3	thoracic level three
T5	thoracic level five
TC	tiny cages
TH	tyrosine hydroxylase
WM	white matter

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Education

March 2014- present	Ph.D. Candidate in Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY
March 2014	MSc. in Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY
May 2011	B.S in Biology minor in Chemistry Georgetown College, Georgetown, KY

Professional Memberships and Activities

2016 – present	American Association of Clinical Anatomists (AACA)
2015 - 2017	Graduate Student Council Representative for the Anatomical Sciences and Neurobiology Department, University of Louisville, Louisville, KY
2014 - 2015	Graduate Student Council Representative Proxy for the Anatomical Sciences and Neurobiology Department, University of Louisville, Louisville, KY
2014 - 2015	Graduate Teaching Academy (GTA) participant

Research experience

Spring 2013-present — Graduate Research Fellow. Dr. David Magnuson Locomotor Systems and Rehabilitation Laboratory, Kentucky Spinal Cord Innovative Research Core

Current research focus: Cardiovascular dysfunction post-spinal cord injury. I am particularly interested in how appropriately timed exercise interventions can influence cardiovascular remodeling following injury. I am also interested in exploring different modalities of exercise (i.e. active vs. passive) of exercise therapies. Other projects include educational research that enhances clinical diagnostic tools incorporated into early medical education.

Fall 2012- 2013	Laboratory Rotation with Dr. Martha Bickford. University of Louisville, KY, Anatomical Sciences and Neurobiology fall 2012
Nov 2011- May 2012	Lab Technician. Dr. Catherine Linnen, Assistant professor, Ecology and Evolutionary Biology; University of Kentucky
June 2010-Aug 2010	Research Assistant. Dr. Patrick Sheridan, Assistant Professor, Organic; Georgetown College, Georgetown, KY

Teaching experience

Spring 2017 Teaching Assistant for Dental Gross Anatomy, University of Louisville, Louisville, KY

Spring 2017 Teaching Assistant for Advanced Head and Neck, University of Louisville, Louisville, KY

Fall 2016 Medical Gross Anatomy Teaching Assistant (with didactic lectures in thorax), University of Louisville, Louisville, KY

Teaching assistants run a dissection zone with four tables, six members per table. Each teaching assistant is also responsible for a lecture component.

Spring 2016 Neurosystems Case Reviewer, University of Louisville, Louisville, KY

Reviewers evaluate and grade cases presented by students during the Medical Neuroanatomy course. They also engage students in discussion relating the relevant neuro-pathways that are disrupted.

Spring 2016 Senior Teaching Assistant for Dental Gross Anatomy, University of Louisville, Louisville, KY

Senior TAs are responsible for in-class dissections and pro-section cadavers. They also mentor junior TAs and aid them with dissection skills and lab preparation.

Spring 2015 Teaching Assistant for Dental Gross Anatomy, University of Louisville, Louisville, KY

The dental gross TA aids first year dental students during all cadaveric dissections. They also prepare pro-dissected cadavers for challenging dissections and exams.

Spring 2015 Tutor for Medical Neurosystems, University of Louisville, Louisville, KY

July 29 2014 Guest lecturer, Fresh Tissue dissection of the Abdomen, University of Louisville, Louisville, KY

July 15 2014 Guest lecturer, Fresh Tissue dissection of the Thorax, University of Louisville, Louisville, KY

Spring 2014 Teaching Assistant for Medical Neurosystems, University of Louisville, Louisville, KY

TA are responsible for aiding students during dissection hours and proctoring tests.

Fall 2013 Teaching Assistant for Medical Neuroanatomy, University of Louisville, Louisville, KY

Spring 2012 Teaching Assistant, Biology lab course 214 Organismal Diversity, Georgetown College, Georgetown, KY

The TA for this course is responsible for setting out organisms investigated during lab up lab. The TA also grade homework and proctored exams.

Publications

DeVeau, K.M., Harman, K.A., Squair, J.W., Krassioukov, A.V., Magnuson, D.S.K., West, C.R. (2016). A comparison of passive hind-limb cycling and active upper-limb exercise provides new insights into cardiac dysfunction following spinal cord injury. **In Review: Am. J of Phys Heart and Circulatory Physiology.**

DeVeau, K.M., Martin, E. K., King, N.T., Shum-Siu, A., Keller B.B., West, C.R., Magnuson, D.S.K. (2016). Challenging Cardiac Function Post-Spinal Cord Injury with Dobutamine. Epub ahead of print**.** Autonomic Neuroscience: Basic and Clinical

Squair, J.W., DeVeau, K.M., Harman, K.A., Poormasjedi-Meibod, M.S., Hayes, B., Liu J., Magnuson, D.S.K., Krassioukov, A.V., West, C.R. (2016). Spinal cord injury induced sympathetic decentralization causes systolic dysfunction and cardiomyocyte atrophy. **In Review: Neurotrauma.**

Harman, K.A., **DeVeau, K.M.**, States, G., King, N.T., Stepp, C., Shum-Siu, A., Magnuson, D.S.K., (2016) Abnormal cardiovascular control during active exercise challenge following incomplete, low thoracic spinal cord injury in rodents. **In Review: Journal of Physiology.**

Harman, K.A., **DeVeau, K.M.**, Wade, A., King, N.T., Wainwright G.N., Shum-Siu, A., Magnuson, D.S.K., (2016) Temporal analysis of cardiovascular control and function at rest and during exercise challenge following incomplete T3 spinal cord injury in rodents. **In Review: Journal of Physiology.**

Presentations *presenting author

DeVeau, K.M.*, Brueckner-Collins, J., Herring, N.R. Using ultrasound to teach FAST exam. American Association of Clinical Anatomists. Oakland, CA, USA. June 2016. Poster Presentation.

Herring, N.R.*, **DeVeau, K.M**., Brueckner-Collins, J. A novel approach for utilizing fresh tissue cadavers to teach the clinical anatomy of the inferior alveolar nerve blcok. Oakland, CA, USA. June 2016. Poster Presentation.

Hayes, B.*, Squair, J.W., **DeVeau, K. M.**, Harman, K.A., Liu, J., Magnuson, D., Krassioukov, A.V., West, C.R. Passive hind-limb cycling is superior to active fore-limb swim training in the restoration of left-ventricular function following upper thoracic spinal cord injury. International Collaboration of Research Discoveries Annual Research Meeting. Vancouver, British Columbia, CA. March 2016, Poster Presentation.

DeVeau, K. M.* Cardiac function following a contusive spinal cord injury. KSCIRC Seminar Series. Louisville, KY, USA. April 2016. Oral presentation.

DeVeau, K. M.*, Harman, K.A., Magnuson, D., Krassioukov, A.V., West, C.R. Assessing Cardiac Function in Experimental Spinal Cord Injury with beta-adrenergic Stimulation and Pressure-Volume Assessments. American Spinal Injury Association. Philadelphia, PA, USA. April 2014. Oral Presentation.

West C.R.***, DeVeau K.M.**, Squair J., Harman K.A., Magnuson D.S.K., Krassioukov A.V. Lesion-dependent impairment in cardiac function after experimental spinal cord injury: insights from left-ventricular catheterization. American Spinal Injury Association. Philadelphia, PA, USA. April 2014. Oral Presentation.

West C.R.***, DeVeau K.M.**, Harman K.A., Squair J., Magnuson D.S.K., Krassioukov A.V. Left-ventricular pressure and volume responses to active- and passive-exercise training following experimental spinal cord injury. experimental biology. San Diego, CA, USA. April 2016. Poster Presentation.

Harman K.A.***, DeVeau K.M.**, Squair J., C.R. West**,** Magnuson D.S.K., Krassioukov A.V. Autonomic Dysreflexia Persists Following Acute Rehabilitation in Rats with Incomplete Contusive Spinal Cord Injury. Experimental Biology. San Diego, CA, USA. April 2016. Poster Presentation.

Squair, J.W.*, **DeVeau, K.M.,** Poormasjedi-Meibod, M., Hayes, B., Harman, M., Liu, J., Magnuson, D., Krassioukov, A.V., West, C.R. Spinal cord injury induces level-dependent impairment in left-ventricular function that is underpinned by alterations in cardiomyocyte structure. 6th Annual ICORD Trainee Symposium, June 2015. Oral Presentation.

DeVeau K.M.*, Martin E.K., King N.T., Shum-Siu A., Tinney J., Keller B.K., Magnuson D.S.K. Challenging cardiac function post-spinal cord injury with Dobutamine. American Spinal Injury Association. Montreal, Quebec, CAN. May 2015. Poster Presentation.

DeVeau K.M.*, Martin E.K., King N.T., Shum-Siu A., Tinney J., Keller B.K., Magnuson D.S.K. Cardiovascular end-organ remodeling in response to SCI and exercise therapy. KSCHIRT Annual meeting. Louisville, KY, USA. March 2015. Oral Presentation.

DeVeau K.M.*, Martin E.K., Brown S.R., Shum-Siu A., Harman K.A., Tinney J., Keller B.K., Magnuson D.S.K.
Using high-resolution ultrasound as a cardiovascular assessment tool post spinal cord injury. Research! Louisville, Louisville, KY. August 2014. Poster Presentation.

DeVeau K.M.*, Martin E.K., Brown S.R., Shum-Siu A., Harman K.A., Tinney J., Keller B.K., Magnuson D.S.K.
Using High-Resolution Ultrasound as a Cardiovascular Assessment Tool Post SCI. KSCIRC Seminar Series. Louisville, KY, USA. June 2014. Oral Presentation.

Awards and Honors

Nov 2014 VISIT Scholarship Award for International Trainees, International Collaboration on Repair Discoveries and University of British Columbia, Vancouver, Canada.

Community Involvement

April 2016 Nano days, Kentucky Science Center, University of Louisville Society for Neuroscience chapter
March 2016 Participant in Convocation of Thanks for the University of Louisville Body Bequeathal program
April 2015 Participant in Convocation of Thanks for the University of Louisville Body Bequeathal program
April 2015 Brain awareness week at the Kentucky Science Cente