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Spasm Intensity and Modulation by Serotonin Across Spinal Cord Injury Severities

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ABSTRACT

Involuntary motor activities such as spasms arise from hyperreflexia in about 70% of individuals with spinal cord injuries (SCI). Despite this prevalence and the negative impact on health and safety, it is unclear what determines the severity of the spasms that develop. This study investigated the impact of injury severity and functional outcome on hyperreflexia intensity in a mouse model of SCI. Hyperreflexia of awake, behaving mice was examined by recording motor outputs *ex vivo* and *in vivo* in response to electrical sensory stimuli. A lack of difference in hyperreflexia between the injury groups and the functional groups suggests that hyperreflexia arising from a complete transection can be just as severe as from a milder SCI. When serotonin (5HT) was added to the *ex vivo* preparation, 5HT increased the intensity of spasms regardless of injury group.

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INTRODUCTION

A. INVOLUNTARY MOTOR BEHAVIORS

Spinal cord injury (SCI) causes paralysis, but also induces changes to reflex function, resulting in involuntary motor behaviors (IMBs), including spasms, in about 70% of individuals with SCI. Spasms are triggered by sensory stimuli and mediated by hyperreflexia to produce strong, multi-joint movements. These movements can interfere with planned motor function and severely impact the quality of life and safety of individuals. It is unclear what determines the spasm severity that develops, or whether the mechanism of spasm development is similar across individuals. Understanding the mechanisms underlying the development of spasms would have a profound effect on the treatment of spasms post SCI, the control of movement, and, therefore, the functional independence of people with SCI.

A.1 TYPES OF IMBs

IMBs are extremely common post SCI and many different presentations have been defined clinically in attempts at describing the IMBs type and severity. Hyperreflexia is defined as overactive or overresponsive reflexes and is an exaggerated motor response to a sensory stimulus. Hyperreflexia mechanisms likely underlie components of other IMBs including spasms. Spasms have been defined as hyperexcitable interneuronal reflexes including multiple joints (Benz et al., 2005). Motor outputs in spasms are long and prolonged and triggered by sensory inputs which are either proprioceptive or exteroceptive. Recent research shows that spasms also require an excitable motoneuron (Murray et al., 2011). Hyperreflexia is also likely involved in spasticity when defined as a velocity-dependent increase in stretch reflexes (Lance, 1980). Another notable IMB post SCI is clonus. Clonus is defined as involuntary rhythmic muscle contractions around a joint at a set frequency of 3 to 8 Hz (Hidler, 1999). Clonus is also a result of sensory input as it is initiated by rapid, but passive stretch of the joint, however, the resultant movement is an alternating movement and not a continuous contraction like in spasms. Another IMB, tone, is also defined by a continuous contraction of muscles, but is not necessarily triggered by a sensory input (O'Sullivan, 2007). Interestingly, there is overlap in the presentation of these IMBs indicating some shared mechanisms, but they have distinct qualities which results in variability in presentation post SCI.

So, although there are common themes, mechanisms of the IMBs are variable and distinct. Future therapies rely on a thorough characterization on how the spinal cord changes post injury with respect to sensory processing as well as motor production.

B. SPINAL MECHANISMS OF HYPERREFLEXIA

The spinal cord is a lattice of information relays. In the coronal plane, sensory information (tactile, thermal, nociceptive, and proprioceptive) is sent from DRG neurons to various local motor circuits. In the sagittal plane descending and ascending axon bundles relay sensory information and motor commands to and from the spinal cord, respectively. Motoneurons integrate all of the graded inputs that they receive to produce a motor output (Heckman et al., 2004). After SCI, the landscape of spinal excitability changes and cause an increase in motor response to normally innocuous sensory inputs. This exaggerated motor response is an important component to many IMBs and has been used to begin to piece out the mechanisms that underlie spasms post SCI (Murray et al., 2011). Most of the research has focused on how motoneuron excitability changes post injury and on which interneuron populations deliver information about the sensory stimulus to the motoneuron. Work by the Bennet lab has used the sacral transection SCI model in mouse and in rat models to investigate the roles of interneurons and in motoneurons in causing hyperreflexia and in causing the long prolonged motor output seen in spasms (Murray et al., 2011). They performed intracellular recordings from motoneurons and whole ventral root recordings in response to dorsal root stimulation in an *ex vivo* sacral cord preparation (Murray et al., 2011). Results from these studies showed that hyperreflexia that leads to spasms depend on 2 mechanisms. First, the motoneuron needs to be excitable enough to be able to respond to incoming excitatory input. Motoneurons also have intrinsic excitability that, when activated, amplify excitatory input delivered and allow motoneurons to fire even when input to the motoneuron is done. Second, the excitatory input to the motoneuron from interneurons needs to be long and strong enough to trigger the endogenous excitability within the motoneuron (Murray et al., 2011).

B.1 INTRINSIC MOTONEURON EXCITABILITY: LLR

Motoneurons are the largest cell type within the spinal cord, perhaps within the central nervous system, and the final determiner of movement. Motoneurons have large dendritic trees that span multiple spinal segments and even have contralateral components and, therefore, constantly integrate an incredible amount of information (Heckman et al, 2004). It was once thought that motoneurons only integrated information and that their output was proportional to the direct input that they receive. The discovery of persistent inward currents (PICs) within the motoneuron completely changed how we understand motor commands are produced (Hounsgaard et al., 1984).

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PIC mechanisms

PICs in motoneurons are activated through metabotropic receptors. Monoamines, such as serotonin, bind to their G-protein linked receptor and stimulate the pathways that activate Na and Ca channels in the dendrites. These channels are voltage-gated and therefore amplify excitatory input to the motoneuron. They are also slow to inactivate and stay open once the current is activated so excitatory input is prolonged even past when the initial stimulus has passed. Motoneurons have 5-HT2c receptors, which are coupled with excitatory Gq proteins activating phospholipase C (PLC)-associated cascades that facilitate calcium and sodium channels in the dendrites of the motoneurons and thereby facilitate PICs (Heckmann, Gorassini, and Bennett, 2005)(Perrier and Hounsgaard, 2003). These PICs amplify and prolong motoneuronal activity, resulting in more frequent firing and providing the potential for continued firing long after the stimulation ends.

Motoneuron response to injury

Initially after SCI, motoneuron excitability is extremely low and motoneuron firing is extremely difficult to initiate. Over time, the motoneuron excitability rises to approximately 80% of normal (Johnson, et al., 2013). This is at least in part due to an increase in proportion of 5-HT2c receptors in the motoneuron that are constitutively active. SCI induces a persistent inflammatory response, activating microglia and downregulating the expression of one of the adenosine deaminases, ADAR2. This reduces mRNA

editing, including that of 5-HT2c which results in a higher percentage of a longer form that is constitutively active. This causes PICs to re-emerge and motoneuron excitability to rise, once again, allowing excitatory input to activate the motoneuron. The PICs are unregulated and always available. Due to the nature of the PIC to amplify motoneuron input to initiate firing and then sustain firing after the input is removed, PICs have been identified as a mechanism underlying spasms (Di Narzo et al., 2015), (Li, Gorassini, and Bennett, 2004).

In total, this exaggerated input to the motor neuron, quantified as the long polysynaptic input (LPR), is strong and long-lasting enough to trigger persistent inward currents (PICs) within the motoneuron. In a study by Murray et al., the Bennet laboratory found that the EPSP that triggers the PICs have to last for around 500 ms and have termed this epoch of response the long polysynaptic reflex (LPR). In their studies, if you decrease the LPR, PICs do not turn on and spasms are not seen. The PIC contribution of the spasm, termed the long-lasting reflex (LLR), is measured from the end of the LPR through the rest of the motor output which can last up to several seconds. In total, these changes in excitability within spinal circuits generates a sustained motor spasm.

B.2 TRIGGER COMPONENTS: LPR

The LPR portion of the spasm is likely composed of inputs from many sources. The interneuron populations described are targets of many investigations.

V3 interneurons

V3 interneurons are genetically identified by their post-mitotic expression of the Sim1 transcription factor (Shirasaki & Pfaff, 2002). They are glutamatergic interneurons distributed throughout the ventral horn (Zhang et al., 2008). The V3 interneuron population has been shown to receive sensory input and the majority of V3 interneurons project contralaterally, with smaller populations shown to project bilaterally or ipsilaterally, onto motoneurons, Ia inhibitory interneurons, and Renshaw cells, allowing them to propagate excitability throughout the cord (Zhang et al., 2008). Based on the complexity of motor behaviors elicited by spasms and V3 interneurons' involvement in locomotor circuitry Bennett's group was interested in the link between locomotor circuits and spasms. They found that optigenetic activation and silencing of V3

interneurons was able to trigger and suppress spasms, respectively both *ex vivo* and *in vivo*. (Lin et al., 2019)

Deep Dorsal Horn interneurons

Laminae III to V make up the most ventral portion of the dorsal horn and is thus called the deep dorsal horn. Recently, the Tysseling lab recorded from DDH interneurons using the *ex vivo* sacral cord preparation in an acutely transected cord and identified several populations of interneurons throughout the DDH based on their firing patterns in response to sensory stimuli: bursting, simple, and tonic (Thaweerattanasinp et al., 2016). The bursting population had a firing pattern that was consistent with a long EPSP input to the motoneuron and was also inhibited by a 5-HT1 agonist, both characteristics that instigate bursting DDH interneurons as part of the spasm trigger (Thaweerattanasinp et al., 2016). In chronic SCI, these bursting DDH interneurons were even more excitable than in the acute injury as noted by an increase of burst duration and a decrease in first-spike latency (Thaweerattanasinp et al., 2020). Zolmitriptan was even more effective in inhibiting the bursting DDH cells by decreasing evoked spike count and increasing first spike latency as compared with acute SCI, which continues to support the hypothesis that these are interneurons involved in spasms. Also, the firing of the bursting DDH had high correlation with the ventral root output. Together, these data show that bursting DDH interneurons are activated with sensory input, have a firing pattern that could add to the EPSP triggering the motoneuron, are sensitive to zolmitriptan which has been shown to decrease EPSP input, and are, therefore likely to play a significant role in spasm generation and prolongation (Thaweerattanasinp et al., 2016 & 2020).

In addition, research from the Heckman lab has shown that motoneuron receptive fields widen after spinal cord injury. In an experiment where they measured from gastrocnemius motoneurons, they could see synaptic input to the motoneuron with ankle movement, but not as much input with knee or hip movement. After SCI, however, gastrocnemius motoneurons would receive just as much input from ankle, knee, and hip movement. Therefore, motoneuron receptor fields greatly widen post SCI so input that would normally affect one joint spreads to the entire hindlimb. This increased spread of sensory information likely also contributes to IMPs (Johnson et al., 2013).

B.3 EFFECT OF SPARED TRACTS ON IMBs POST INCOMPLETE INJURY.

It is clear that there is much to learn regarding mechanisms that underlie IMBs. One additional area that has been overlooked is how spared fibers alter spinal excitability across the spinal cord. Most mechanistic work has been done after transection injury so that we can specifically identify excitability changes across the cord in response to injury, but because most human SCI has spared tracts, we need to understand how this can alter excitability states.

Serotonin's role in IMBs

As noted above, the serotonergic receptor system is quite involved in hyperreflexia and spasms. Constitutively active 5-HT2c receptors in sacral motoneurons are at least in part responsible for the intrinsic excitability of the motoneuron and its ability to fire and have prolonged firing post SCI (Murray et al., 2011). Disinhibition of the DDH interneurons that express 5HT1b/d has been shown to contribute to the trigger for hyperreflexia (Murray et al., 2011). The presence of the serotonin ligand through spared raphespinal tracts may also greatly affect the landscape and may do so with respect to the prevailing neuron type contributing to the IMB (Murray et al., 2011).

The raphespinal tracts originate in the three most caudal raphe nuclei, the raphe magnus, the raphe obscurus, and the raphe pallidus and is the main source of 5-HT in the spinal cord (Carlsson, Magnusson & Rosengren, 1963). These three nuclei supply two diffuse descending tracts that run through the ventrolateral funiculus. The raphe magnus supplies the dorsal regions of the spinal cord while the raphe obscurus and pallidus innervate the ventral regions (Tanaka et al., 2006). The effect of 5-HT in the spinal cord depends on the 5-HT receptors expressed on the neurons in the spinal cord. For example, 5-HT2c receptors not only become constitutively active post SCI, but they also become 40x supersensitive to serotonin. Spared raphespinal tracts to the motoneuron would increase excitability in the final motor output. Alternatively, the lack of serotonin to the deep dorsal horn induces disinhibition in the 5HT1b/d-expressing bursting DDH interneurons that contribute to hyperreflexia/spasms. If serotonin is spared to these interneurons, inhibition is restored and the excitatory trigger to the motoneuron is lessened. Other interneuron populations may also be affected. For example, the glutamatergic V2a interneurons identified by their expression of Chx10 have been shown to contribute to left-right alteration and those that

coexpress Shox2 synpase directly onto motoneurons. V2a interneurons have been shown to have no general increase in excitability after spinal cord injury, but are 1000x more sensitive to serotonin following SCI (Husch et al., 2012) . This means that when measuring excitability across the cord in a transection model, the V2a interneurons do not show a change, but if spared raphespinal tracts exist, the V2a interneurons would be much more excitable. In summary, the extent to which the different receptor subtypes are affected post injury in addition to the amount of spared serotonin present to those receptors may be a large contributor to the presence and presentation of IMBs post SCI. Uncovering these changes and how they relate to IMBs will dramatically change how we view and treat people with SCI.

C. IMBs in Human

There is much contention as to whether spasms in humans vary with injury severity. There are studies that find a positive correlation between injury severity and those that find no correlation. These studies use self reporting measures rather than directly measuring the IMBs. Studies that are investigating human IMBs following SCI are revealing some interesting trends. First, it has been shown in humans that spasms are ameliorated via zolmitriptan administration suggesting that similar spasm triggers exist in both humans and rodents (D'Amico et al., 2013). Second, it has been shown in humans that motor incomplete injuries have more severe spasticity than motor complete injuries (Sangari et al., 2019). Amongst those motor incomplete injuries, by testing how startle responses modulate motor output, this group has also found that the ratio of corticospinal tract to reticulospinal tract (CST/RST) sparing significantly contributes to the intensity of spasticity (Sangari et al., 2019). When looking at subchronic timepoints they have found that differences in spasticity between motor impaired groups are more pronounced at these earlier timepoints (Sangari et al., 2022). Taken together, the findings in humans suggest that similar mechanisms of IMBs are present in humans and in rodents and that in humans spared descending fibers augment the spinal changes depending on the tracts that are spared.

D. PURPOSE OF THESIS

In animal studies the neuronal mechanisms behind spasms have been teased apart in a complete transection model of SCI using cellular recording strategies while in human studies the impact

of spared fibers on the intensity of IMBs is being elucidated. In the animal model it is unknown how spared fibers in an incomplete injury model impact spasm behavior and direct recording from human spinal neurons is unavailable. Thus it is unknown how spasm intensity interacts with the spared fibers in an incomplete injury. To acquire this information these studies examine the effect of incomplete injury on spasm intensity, observing how spasms present themselves in different severities of injury in a mouse model of SCI. Results from this work will elucidate potential subpopulations of spasms and differences in the contribution of mechanisms to the intensity of spasms observed. The results of this study have implications for therapeutic targets and diagnostic tools for sufferers of spasms following SCI.

CHAPTER 1

Introduction

Spinal cord injury (SCI) induces changes to reflex function, resulting in involuntary motor behaviors, including spasms, in about 70% of individuals with SCI (Levi, Hulting, and Seiger, 1995). Spasms are triggered by sensory stimuli and mediated by hyperreflexia to produce strong, multi-joint movements (Rabchevsky and Kitzman, 2011). These movements can interfere with function and impact independence. It is unclear what determines the severity of spasms or whether the mechanisms are similar across individuals. This knowledge would benefit the treatment of spasms, the control of movement, and improve function post SCI.

Endogenous motoneuron activity, particularly persistent inward currents (PICs), is key for hyperreflexia. PICs are long-lasting influxes of cations that make the motoneuron more excitable and capable of persistent firing (Hounsgaard et al., 1984). These PICs are turned on by activity in many interneuronal populations which are triggered by sensory input (Bellardita et al. 2017), (Borowska et al., 2013), (Lin et al., 2019), (Thaweerattanasinp et al., 2016), (Thaweerattanasinp et al., 2020). Bursting deep dorsal horn interneurons become hyperexcitable post injury and relay sensory information to the motoneurons (Thaweerattanasinp et al., 2016 & 2020). The V3 interneuronal population has been shown to receive sensory input and to have bilateral arborization, allowing them to propagate excitability throughout the cord(Borowska et al., 2013), (Lin et al., 2019), (Blacklaws et al., 2015), (Chopek et al., 2018), (Rybak, Dougherty, and Shevtsova, 2015), (Zhang et al., 2008). In addition, motoneuronal receptor fields widen post SCI such that interneuronal input spreads to the entire hindlimb (Johnson et al., 2013). In combination, this exaggerated input to the motor neuron delivers a long excitatory postsynaptic potential to an excitable motoneuron (Murray et al., 2010), (Murray et al., 2011) which is strong and long-lasting enough to trigger PICs within the motoneuron. In total, these changes in excitability generate hyperreflexia and a sustained motor spasm (Murray et al., 2010).

The severity of hyperreflexia that develops is variable and the mechanisms underlying the severity of hyperreflexia are unknown. One uninvestigated possible mechanism is that hyperreflexia may be related to injury severity or functional recovery. Most mechanisms were discovered using sacral transection injuries, but the majority of human SCI is anatomically incomplete so the translation of this knowledge to human SCI is mostly untested (Levi, Hulting, and Seiger, 1995). Recent studies in rodent models of incomplete injury show a continued role of motoneuronal excitability in hyperreflexia (Tysseling et al., 2017), but the extent to which incompleteness of injury, and therefore the presence of spared fibers, affects the hyperreflexia is still unknown. In general, human studies have shown varying effects depending on timing, testing, and type of hyperreflexia (Holtz et al., 2017), (Little et al., 1989), (Richard-Denis et al., 2020), (Skold, Levi, and Seiger, 1999), (Skoog and Jakobsson, 2020), (Sangari and Perez, 2022).

The current study is a first step to investigating how hyperreflexia and its underlying mechanisms vary with injury severity and with functional outcome. We tested hyperreflexia *ex vivo* and *in vivo* by recording motor outputs in response to a sensory input. Our results show that the development of hyperreflexia is not different comparing transection and incomplete injury or comparing functional outcomes with incomplete injury. In addition, neither motoneuronal triggers nor endogenous motoneuronal excitability are different across injury severity or across functional outcomes. Interestingly, hyperreflexia in the sacral region doesn't necessarily predict hyperreflexia in the lumbar region. Results from this study have important implications for the use of hyperreflexia as a biomarker for recovery as well as for the understanding mechanisms for future treatments.

Methods

Spinal cord injuries and mouse grouping- Mice were anesthetized with isofluorane and a laminectomy was performed at the T11 spinal level. In the complete transection group (cTX, n=14), a full transection was performed by cutting the spinal cord with spring scissors. The incomplete impact group (iMP) received incomplete injuries via the Infinite Horizons Spinal Cord Impactor System (IH-0400 Precision Systems and

Instrumentation) with a 70kdyne force and 60sec dwell. Using the Basso Mouse Scale (BMS), these mice were scored at 10 weeks post injury and then grouped according to those scores as the degree of spared locomotor function has been shown to correlate with tissue damage (Cao et al., 2005), (Scheff et al., 2003), (Schucht et al., 2002). The groups were delineated as: BMS score ≤ 3 were the incomplete non-weight-bearing (iNWB, n=10) group, BMS score between 3 and 6 were the incomplete weight-bearing (iWB, n=12) group, and BMS score > 6 were the incomplete coordinated stepping (iCO, n=8) group.

Ex vivo sacral cord preparation- This preparation has been previously published (Jiang and Heckman, 2006). Briefly, mice were anesthetized with urethane (.54mg/kg) and the sacral section of the spinal cord was removed. S1-S3 dorsal roots were mounted to stimulating hooks and the ventral roots to recording hooks. The dorsal roots were stimulated at 3x afferent threshold intensity with a 5-train 1msec stim with a 10msec interspike interval every minute. Reflex excitability in isolated sacral spinal cords was generally low because of the acute spinal shock. Therefore, low doses of strychnine (1 μM) and picrotoxin (10 μM) were applied to the recording. Electroneurograms (ENGs) of the last 5 responses from the 6 recorded sacral roots were amplified (100×) and band-pass filtered (300–10,000 Hz) before being digitized (20 kHz) for analysis.

Flexor withdrawal- This technique was previously published and adopted from human research (Tysseling et al., 2017), (Hornby et al., 2004). Mice were anesthetized with isoflurane, and fine-wire recording electrodes were inserted into the tibialis anterior (TA) and lateral gastrocnemius (LG) muscles. Stimulating ball electrodes were then attached to the plantar hindpaw. Once the mouse was awake, trains of 5 square pulses, 1msec duration stimuli with 10msec intervals were delivered 2 minutes apart with an amplitude determined by the 5x threshold for visible leg movement, inducing a flexor withdrawal reflex recorded with EMGs. Only trials for which there was no substantial EMG activity in all muscles before the stimulation were included for subsequent analyses (3 trials per mouse). EMGs from the leg ipsilateral to the stimulation electrode were amplified (A-M Systems, model 3500; 10,000×) and band-pass filtered (30–1,000 Hz) before being recorded to computer (5,000 Hz) for off-line data analysis.

Spasm epochs- To quantify the spasm intensity, the integral over several durations of the rectified EMG/ENG was calculated. These epochs correspond to motoneuronal firing due to different inputs as

defined previously (Murray et al., 2011). *Ex vivo*, the signal was rectified and integrated over 1 and 10 seconds of LLR (LLR1, LLR10) and *in vivo* signals were integrated over 1 and 3 seconds of LLR (LLR1, LLR3). The baseline electrical activity 1 sec before the stimulus was rectified and a comparable multiple of the integral was subtracted from the response epochs. These two time points were chosen to both examine the highest intensity of LLR(LLR1) and have a measure that corresponded to duration (LLR10/3) (average responses were 8 seconds long *ex vivo* and 1.5 seconds *in vivo*). Reflex magnitude was calculated as rectified, integrated EMG/ENG (riENG/riEMG).

Statistical Analysis- This work was exploratory in nature, rather than confirmatory, and therefore no formal power analysis was conducted. Density plots were generated to explore the distribution of outcomes of interest. Side-by-side boxplots were generated across injury group, channel, and muscle to explore the difference between groups. Generalized linear mixed effect models were fit on the log transformed outcomes with a random intercept for mouse and stimulation within mouse as well as fixed effects for injury group, muscle, and the interaction term between injury group and muscle. ANOVA was conducted to test whether the interaction term was statistically significant. For *in vivo* data, generalized linear mixed effect models were also fit separately for TA and LG, with a random intercept for mouse as well as fixed effects for injury group. The correlation of outcomes of interest between *in vivo* and *ex vivo* data was explored through generalized linear mixed effect models with a random intercept for mouse and stimulation within mouse as well as fixed effects for which dataset the outcome originates, and injury group. ANOVA were conducted to test whether the injury group significantly improves the model fit. Average values of outcomes for both *in vivo* and *ex vivo* were calculated for each mouse across channels. Scatterplots of outcomes were generated to show the patterns between *in vivo* and *ex vivo*.

Measures of cocontraction were calculated for all spasm epochs. Generalized linear mixed effect models were also fit on the log transformed measures of cocontraction, with a random intercept for mouse as well as fixed effects for injury group.

Results

Table 1 lists the group descriptive statistics for all of the data described in this section.

				Functional/Injury Group			
				cTX	***iMP***	***iNWB***	***iWB***	***iCO***
Ex vivo sacral cord preparation		LPR riENG (mV)	*mean*	0.04597	0.04604	0.04708	0.04705	0.04414
			s.e.m.	0.001021	0.0008619	0.001419	0.001468	0.00154
		1 second LLR riENG (mV)	*mean*	0.09026	0.09149	0.09267	0.09475	0.08777
			s.e.m.	0.00198	0.001698	0.002736	0.002882	0.00311
		10 second LLR riENG (mV)	*mean*	0.3949	0.3547	0.3743	0.3819	0.313
			s.e.m.	0.01041	0.009275	0.01671	0.01661	0.01405
		n(roots)		80	126	51	35	40
		n(mice)		14	23	9	6	8
In vivo flexor withdrawal	Tibialis Anterior (TA)	LPR riEMG (mV)	*mean*	0.001665	0.000825	0.002356	0.002057	0.00256
			s.e.m.	0.00016	9.55E-05	0.000226	0.000217	0.00044
		$\log_{10}$ LPR riEMG (mV)	*mean*	-2.88399	-2.7655	-2.71085	-2.79174	-2.80484
			s.e.m.	0.045101	0.03832	0.048876	0.053589	0.11106
		1 second LLR riEMG (mV)	*mean*	0.001022	0.000928	0.001776	0.001381	0.00164
			s.e.m.	0.0002	0.000252	0.000447	0.000397	0.00041
		$\log_{10}$ 1 second LLR riEMG (mV)	*mean*	-3.53087	-3.22619	-3.19039	-3.25555	-3.23344
			s.e.m.	0.13087	0.076477	0.117655	0.109588	0.18588
		3 second LLR	*mean*	0.001908	0.002116	0.003179	0.002547	0.00292

Lateral Gastrocnemius (LG)	riEMG (mV)	*s.e.m.*	0.000372	0.000641	0.000931	0.000984	0.00088
	log_{10} 3 second LLR riEMG (mV)	*mean*	-3.21473	-2.95337	-2.9904	-3.00083	-2.81253
		s.e.m.	0.121539	0.071166	0.124585	0.110259	0.1380
	LPR riEMG (mV)	*mean*	0.000825	0.0007754	0.0007277	0.0007085	0.0009497
		s.e.m.	9.55E-05	7.60E-05	9.95E-05	0.0001137	0.0001998
	log_{10} LPR riEMG (mV)	*mean*	-3.24055	-3.30491	-3.30831	-3.37858	-3.18054
		s.e.m.	0.057209	0.046851	0.079832	0.081928	0.073252
	LLR1 riEMG (mV)	*mean*	0.000928	0.0004712	0.0007625	0.0002337	0.0004567
		s.e.m.	0.000252	0.0001261	0.000345	7.41E-05	0.000106
	log_{10} LLR1 riEMG (mV)	*mean*	-3.79698	-3.85238	-3.87054	-4.01159	-3.57102
		s.e.m.	0.153259	0.077383	0.142121	0.105806	0.111888
	LLR3 riEMG (mV)	*mean*	0.002116	0.0009905	0.001632	0.0005952	0.0007501
		s.e.m.	0.000641	0.0003066	0.0008503	0.0001971	0.0001641
	log_{10} LLR3 riEMG (mV)	*mean*	-3.39647	-3.56112	-3.63266	-3.67977	-3.27154
		s.e.m.	0.145619	0.076767	0.152196	0.119465	0.094156
	n(mice)		17	32	11	13	8
LPR TA/LG ratio		*mean*	3.961	5.988	6.001	6.559	5.042
		s.e.m.	1.005	1.014	1.641	1.794	1.933
log_{10} LPR TA/LG ratio		*mean*	0.356559	0.570339	0.682222	0.586838	0.375702

In vivo cocontraction						
	s.e.m.	0.065419	0.053233	0.090392	0.068227	0.127186
LLR1 TA/LG ratio	*mean*	7.684	9.581	11.78	10.89	3.706
	s.e.m.	3.885	2.062	4.123	3.259	1.622
log_{10} LLR1 TA/LG ratio	*mean*	0.289929	0.707144	0.803257	0.815389	0.386397
	s.e.m.	0.16089	0.082073	0.142117	0.095723	0.200733
LLR3 TA/LG ratio	*mean*	7.849	10.03	12.9	10.72	4.219
	s.e.m.	6.364	2.574	5.252	4.152	1.593
log_{10} LLR3 TA/LG ratio	*mean*	0.170693	0.66081	0.800704	0.695678	0.410064
	s.e.m.	0.153595	0.089153	0.155544	0.117022	0.19935
n(mice)		16	31	11	13	7

Table 1 descriptive statistics for the injury and functional outcome groups.

Severity of hyperreflexia ex vivo does not vary with injury severity or functional outcome.

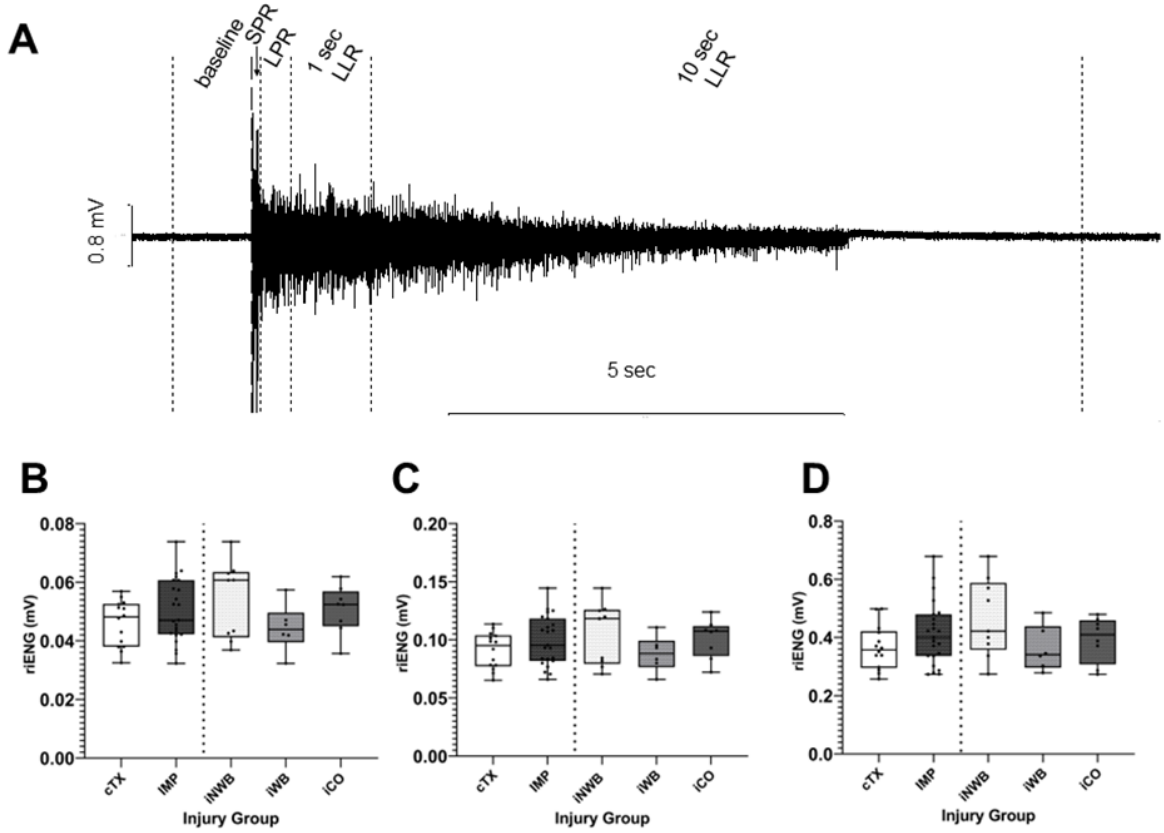
A
baseline
SPR
LPR
1 sec
LLR
10 sec
LLR
0.8 mV
5 sec
B
C
D
riENG (mV)
cTX
iMP
iNWB
iWB
iCO
Injury Group

Figure 1.1 Ex vivo measurements of fictive spasms show no difference between injury groups or functional outcomes. (A) Example ENG trace of ventral root response to 3xT stimulation to dorsal root. Vertical hashed lines denote 1 second of baseline activity, short polysynaptic reflex (SPR), long polysynaptic reflex (LPR), 1 and 10 seconds of long-lasting response (LLR). Large, hashed lines represent stimulus onset. The average response lasted for approximately 8 seconds. Subfigures B, C, and D display box-and-whisker plots of the per mouse average rectified integrated ENG (riENG) ventral root responses during the LPR, 1 second of LLR, and after 10 seconds of LLR, respectively. Mice are grouped by injury type (transection (cTX),white and impact (IMP),dark gray, spotted) on the left subgraph and the impact group is further subdivided by functional outcomes on the right(non-weight bearing (iNWB), light gray, weight-bearing (iWB), gray, and coordinated (iCO), dark gray). Dots represent average ventral root responses from individual mice.

The cTX group LPR, 1 second LLR, and 10 second LLR were not different from the impact group (LPR(p=0.9994), 1 second LLR(p=0.8857), 10 second LLR (p=0.3344) nor the iNWB group with similar locomotor function (LPR (p=0.8582), 1 second LLR (p=0.8417), 10 second LLR (p=0.706). There was no significant difference in ventral root output amongst the impact functional groups LPR(iNWBxiWB p=0.9993, iNWBxiCO p=0.8811, iWBxiCO p=0.8867), 1 second LLR (iNWBxiWB p=0.9759, iNWBxiCO p=0.9196, iWBxiCO p=0.8439), 10 second LLR (iNWBxiWB p=0.991, iNWBxiCO p=0.6376, iWBxiCO p=0.617)(mixed model ANOVA, posthoc Tukey). Descriptive statistics for all groups are listed in Table 1.

Ex vivo hyperreflexia was tested through the sacral cord preparation. Figure 1A shows a representative example of an *ex vivo* root recording with the two different stages of spasm. We tested the effect of injury type by comparing the cTX group to the IMP group (cTXxIMP: t=.001, p= 0.999, Student's t-test) and to the motor function-matched iNWB group (cTXxiNWB t=.181, p=0.858, Student's t-test). Neither the IMP group, nor the iNWB group were different from the cTX group in the LPR phase[6], which shows the motor response due to synaptic input to the motoneuron. The LLR phase, which measures motoneuronal PICs

(LLR1 (cTXxIMP: t=0.145, p= 0.9994, cTXxiNWB: t=0.202, p= 0.8582, Student's t-test), LLR10(cTXxIMP: t= -0.979, p= 0.3344, cTXxiNWB: t= -0.382, p= 0.6739, Student's t-test)) similarly was not different at 1 or 10 seconds (Fig1C-D, left graph). Ventral root output was also not different when comparing the functional outcome groups, iNWB, iWB, and iCO, in the LPR phase (iNWBxiWB: F=.037 p=0.971, iNWBxiCO: F=-0.481, p=0.636, ANOVA) (Figure 1B, right graph). Similarly, there were no differences in the LLR phase, in either the LLR1 (iNWBxiWB: F= 0.210, p= 0.835, iNWBxiCO: F=-0.391 p= 0.700, ANOVA) or in the LLR10 (iNWBxiWB: F= 0.128, p= 0.900, iNWBxiCO: F=-0.915, p=0.371, ANOVA) (Figure 1C-D, right graph). Group descriptives statistics can be found in Table 1. In total, there was no *ex vivo* difference in overall synaptic input to the motoneuron or endogenous motoneuron excitability across injury severities.

Severity of hyperreflexia in vivo does not vary with injury severity or functional outcome.

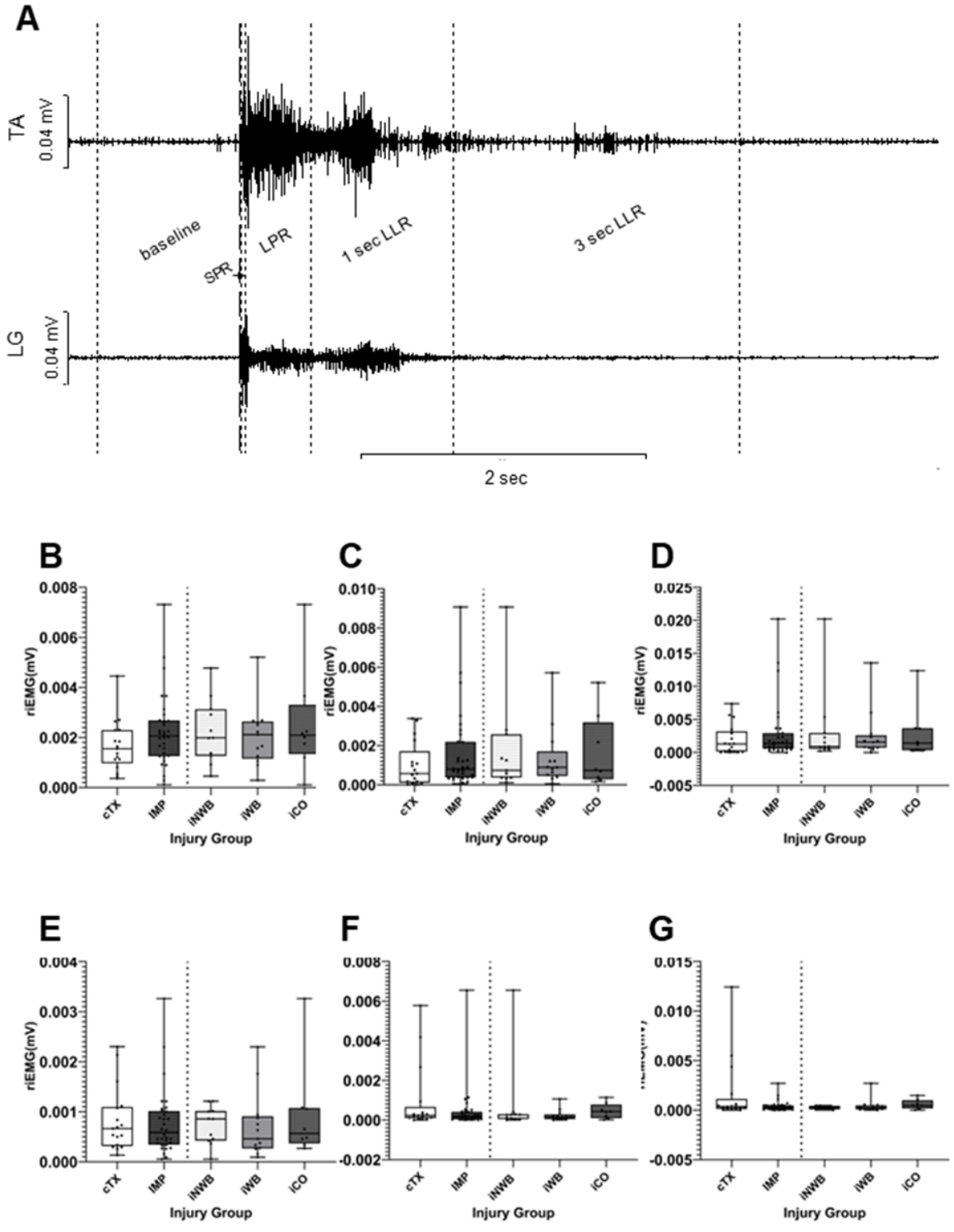
A
TA
0.04 mV
baseline
SPR
LPR
1 sec LLR
3 sec LLR
LG
0.04 mV
2 sec
B
C
D
E
F
G
riEMG(mV)
Injury Group
cTX
iMP
iNWB
iWB
iCO

Figure 1.2 *In vivo* measurements of spasms show no difference between injury groups or functional outcomes. (A)Example EMG trace of flexor withdrawal response of tibialis anterior (TA) (top) and lateral gastrocnemius (LG) (bottom) to plantar paw electrical stimulation. Vertical dotted lines bound 1 second of baseline EMG activity, the SPR, LPR, and 1 and 3 seconds of LLR. Graphs (B-F) are formatted as graphs in Figure 1. Panels B-D display data from TA muscles while panels E-G display LG muscle data. The cTX group was not different from the impact group (TA(LPR (0.2533, Student's t), 1 second LLR (0.1033, Student's t), 3 second LLR (0.1272, Student's t), LG(LPR(0.6314, Student's t), 1 second LLR(0.9613, Student's t), 3 second LLR(0.8687, Student's t)) nor the impact mice with similar function (TA(LPR (0.1371, t-test), 1 second LLR (0.2093, t-test), 3 second LLR (0.3433, t-test), LG(LPR(0.6743, Student's t), 1 second LLR (0.9222 , Student's t), 3 second LLR (0.7551 , Student's t)) . There was no difference amongst the impacted functional groups (TA (LPR(iNWBxiWB: 0.8623, iNWBxiCO: 0.8495, iWBxiCO: 0.9956), 1 second LLR (iNWBxiWB: p-value, iNWBxiCO: 0.984, iWBxiCO: 0.9825) 3 second LLR(iNWBxiWB:0.9927 iNWBxiCO: 0.8005, iWBxiCO: 0.8542), (LG(LPR (iNWBxiWB: 0.9174, iNWBxiCO 0.7623, iWBxiCO: 0.5297), 1 second LLR (iNWBxiWB: 0.8477, iNWBxiCO: 0.5135, iWBxiCO: 0.244), 3 second LLR (iNWBxiWB: 0.9857 iNWBxiCO: 0.4261, iWBxiCO: 0.3305)mixed model ANOVA)). Descriptive statistics for all groups can be found in Table 1.

The *in vivo* results demonstrate systematic hyperreflexia which incorporates spinal excitability and spared fiber effects. Figure 2A shows a representative example of the TA and LG responses to a brief plantar stimulation after a transection injury. Note the long motor response to a brief electrical stimulus. *In vivo* hyperreflexia severity also shows no difference across injury types during the LPR (TA (cTXxIMP: t= 1.156, p= 0.2533, cTXxiNWB: t= 1.532, p= 0.1371), LG (cTXxIMP: t= -0.483, p= 0.6314, cTXxiNWB: t= -0.425 p= 0.6743, Student's t-test)) (Figure 2B, 2E, left graph), LLR1 (TA (cTXxIMP: t= 1.662, p= 0.1033 , cTXxiNWB: t= -0.099, p= 0.2093), LG (cTXxIMP: t= -0.049, p= 0.9613, cTXxiNWB: t= -0.099, p= 0.9222, Student's t-test)) (Figure 2C, 2F, left graph) and LLR3 (TA (cTXxIMP: t=1.553, p= 0.1272 , cTXxiNWB:

t=0.965, *p*= 0.3433), LG (cTXxIMP: *t*=-0.166, *p*= 0.8687, cTXxiNWB: *t*= -0.315, *p*= 0.7551, Student's t-test)) (Figure 2D, 2G, left graph). Likewise, there was no significant difference in hyperreflexia amongst the functional outcome groups (LPR (TA (iNWBxiWB: *F*= -0.520, *p*= 0.607, iNWBxiCO: *F*= -0.546, *p*= 0.589), LG (iNWBxiWB: *F*= -0.396, *p*= 0.695, iNWBxiCO: *F*= 0.706, *p*= 0.486)) (Figure 2B, 2E, right graph), LLR1 (TA (iNWBxiWB: *F*= .008, *p*= 0.994, iNWBxiCO: *F*= -0.171, *p*= 0.885), LG (iNWBxiWB: *F*= -0.549, *p*= 0.587, iNWBxiCO: *F*= 1.114, *p*= 0.275)) (Figure 2C, 2D, right graph), LLR3 (TA (iNWBxiWB: *F*= 0.116, *p*= 0.909, iNWBxiCO: *F*= 0.639, *p*= 0.528), LG (iNWBxiWB: *F*= -0.162, *p*= 0.873, iNWBxiCO: *F*= 1.265, *p*= 0.216, ANOVA)))(Figure 2D, 2G, right graph). Figures 2B-2G show that the development of hyperreflexia does not differ between mice with better or worse functional outcomes.

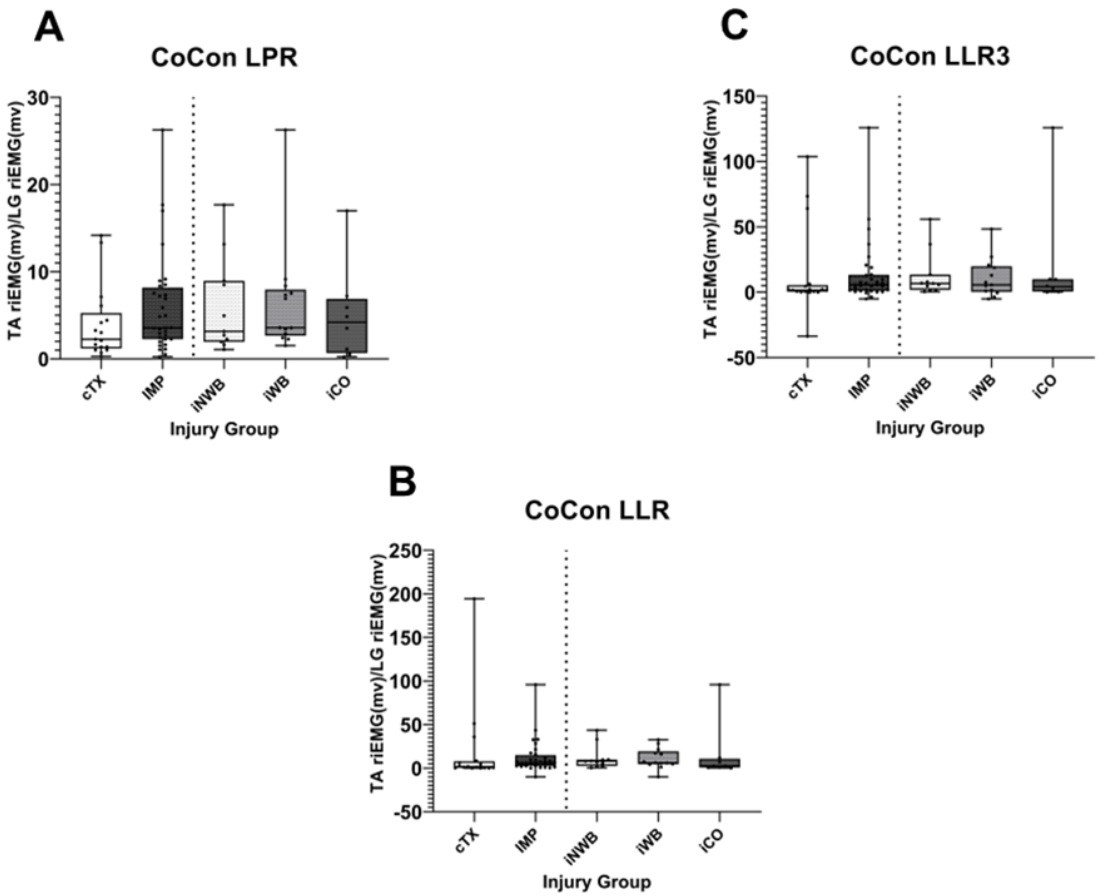

Figure 1.3 Degree of cocontraction across injury and functional groups. Box-and-whisker plots of the ratio of riEMG TA and LG (TA/LG). ratio values for the LPR (A), 1 second (B) and 3 seconds (C) of LLR are presented in a similar layout to Figures 1 and 2. Dots represent ratio values from individual mice. The cTX group was not different from the impact group (LPR 0.18062, 1 second LLR 0.1235, 3 second LLR 0.1101), nor the iNWB group (LPR 0.18278, 1 second LLR 0.2778, 3 second LLR 0.1855). The impact injury groups were not significantly different (LPR (iNWBxiWB p= 0.9973, iNWBxiCO p=0.5736, iWBvsiCO p=0.5138), 1 second LLR(iNWBxiWB p=0.8161, iNWBxiCO p=0.6189, iWBvsiCO p=0.294), 3 second LLR (iNWBxiWB p=0.9951, iNWBxiCO p=0.6299, iWBvsiCO p=0.5589, ANOVA). Descriptive statistics for all groups can be found in Table 1.

Another commonly used test for spasms examines the cocontraction of antagonistic muscles. We quantified the extent to which TA and LG were activated in the LPR period (cTXxIMP: t= 1.359, p= 0.18062 , cTXxiNWB: t= 1.369, p=0.18278, Student's t-test) (iNWBxiCO: F= 0.070, p=0.319, iNWBxiCO: F= -1.015, p=0.945, ANOVA)(Figure 3A) LLR1 (cTXxIMP: t= 1.569, p= 0.1235, cTXxiNWB: t= 1.109, p= 0.2778, Student's t-test iNWBxiWB: F= 0.610, p= 0.35391 iNWBxiCO: F= -0.942, p= 0.54671, ANOVA) (Figure 3B) and LLR3 (cTXxIMP: t= 1.629, p=0.110, cTXxiNWB: t= 1.361, p=0.186, Student's t-test, iNWBxiWB: F= 0.094, p=0.36317, iNWBxiCO: F= -0.925, p=0.92543, ANOVA) (Figure 3C). We again found that the activation ratio of the TA vs LG did not differ across injury type or functional outcome in either the LPR or LLR period. These results, along with the integrated EMG measurements, support the thesis that neither functional outcome nor injury type determines the overall development of spinal changes underlying hyperreflexia or abnormal muscle coupling.

Hyperreflexia may not correlate across spinal segments.

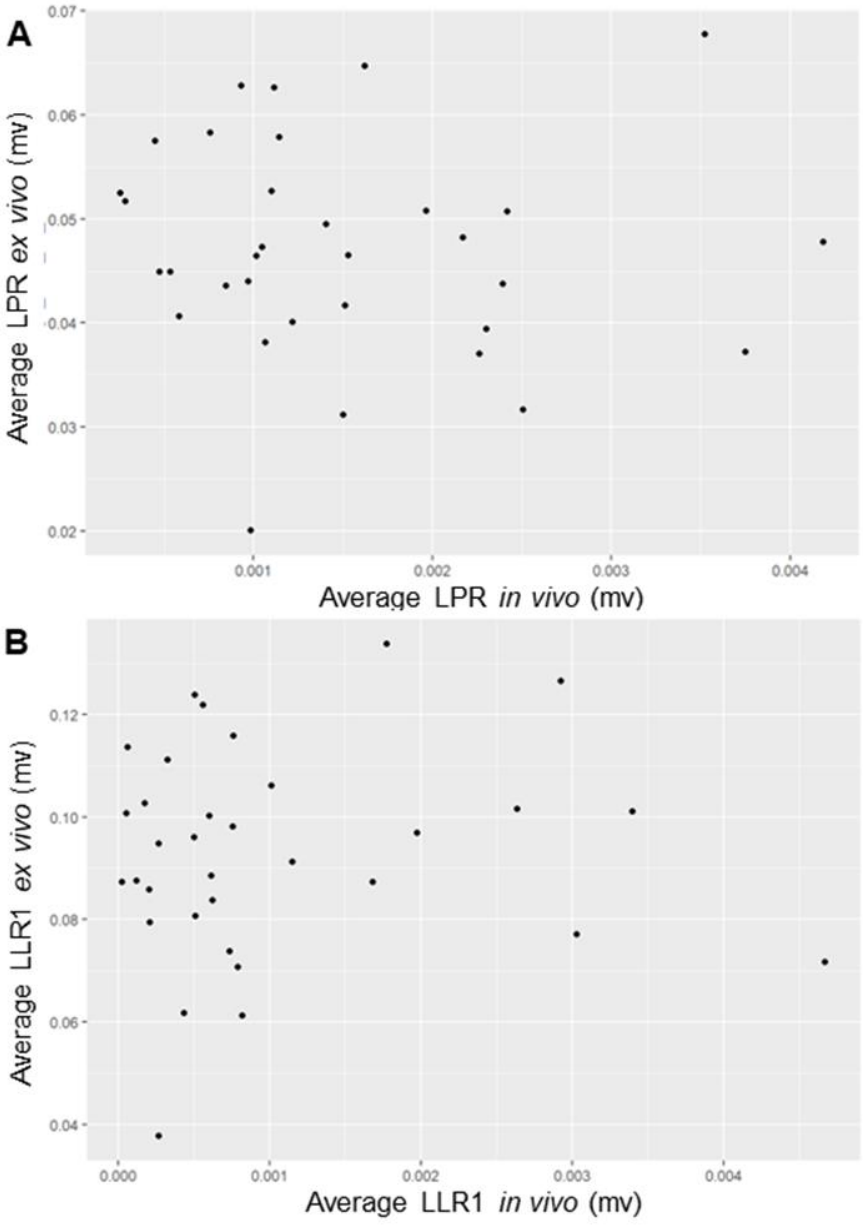
A
Average LPR ex vivo (mv)
0.07
0.06
0.05
0.04
0.03
0.02
0.001
0.002
0.003
0.004
Average LPR in vivo (mv)
B
Average LLR1 ex vivo (mv)
0.12
0.10
0.08
0.06
0.04
0.000
0.001
0.002
0.003
0.004
Average LLR1 in vivo (mv)

Figure 1.4 Correlation of in vivo and ex vivo LPR (A) and 1 second of LLR (B). *In vivo* flexor withdrawal riEMG values are charted on the x-axis and ex vivo riENG values are charted on the y-axis. The correlation between these values is weak (A (LPR): r=-0.668814, *p*=0.7115, B (LLR1): r=0.0587011, *p*=0.7456 (Pearson's correlation))).

The *in vivo* flexor withdrawal has a similar sensory input and a similar motor output as the *ex vivo* cord preparation but tests hyperreflexia in a different spinal segment, the lumbar spinal cord. When comparing the average hyperreflexia in the two tests in the same mouse, the results show that hyperreflexia is not correlated (LPR: r=-0.668814, p=0.7115, LLR1: r=0.0587011, p=0.7456 (Pearson's correlation)) (Figure 4A-B).

Discussion

Most of what is known about how spinal circuitry changes post SCI has been done in a complete transection model looking at sacral changes, but incomplete injury, which is far more prevalent in human SCI, has not been thoroughly investigated, particularly mechanistically. This study asks whether hyperreflexia and its underlying mechanisms vary with injury severity and with functional outcome. Both our *in vivo* and *ex vivo* data show that hyperreflexia can be just as severe regardless of injury severity or functional outcome. One explanation could be that mechanisms underlying hyperreflexia are weighted more toward spinal changes as adding spared fibers in the *in vivo* approach did not change the overall result. The development of hyperreflexia may be dependent on an on/off mechanism triggered by SCI, but not modulated by the degree of injury, such as a trigger in an inflammatory pathway. Alternatively, another explanation for this result could be that the various contributing neuronal groups may have different spinal neuronal excitation changes post injury, resulting in variable hyperreflexia. Fluctuations in the extent to which individual neuronal populations change is unknown and may be synergistic or antagonistic, contributing to this variability. Our data are consistent with this explanation as mice within each functional outcome category show a variety of hyperreflexia intensities. Current studies in our lab are examining the specific role of contributing mechanisms such as individual currents or intrinsic motoneuronal excitability to elucidate their specific roles in hyperreflexia. Defining these specific mechanisms will allow for better therapeutics to control hyperreflexia while allowing functional volitional movement.

An important point is that our *in vivo* and *ex vivo* results both show no difference in hyperreflexia among functional outcomes. The *ex vivo* condition, as fictive movement, is straight forward with respect to the stimulus delivered and the time points measured in LPR and LLR. *In vivo* testing is inherently more variable. Leg movements can cause additional sensory input throughout the testing, possibly making the LPR, and LLR time points more fluid and variable. Another variable in the *in vivo* testing is the possibility of volitional movement confounding the results. Despite these issues, we believe our results to be valid and reliable for several reasons. First, mice in SCI studies are handled daily and as a result are tame and quiescent during testing so volitional input, although it may exist, does not dominate the response. In addition, uninjured control records show a short, high amplitude response which is a very different pattern that we see in our injured mice. Finally, our *in vivo* results are overall consistent with our *ex vivo* results. Surprisingly, the addition of spared tracts *in vivo*, which should change the dynamic within the spinal cord, did not change this finding.

Despite the converging *in vivo* and *ex vivo* hyperreflexia results, individual mouse results in the sacral *ex vivo* preparation and the *in vivo* lumbar tests were not positively correlated. One likely explanation of this is that the amount of hyperreflexia in the sacral cord does not necessarily match the hyperreflexia in the lumbar cord. Additionally, *in vivo* results can also reflect the state of the animal which may have an effect on the overall result. Alternatively, the correlation of the *ex vivo* and *in vivo* results may be masked by the contribution of spare fibers in mice with less severe injuries.

As stated in the introduction, results in human data are variable. Several human studies examining spasticity or hyperreflexia indicated that persons with motor incomplete and cervicothoracic injuries reported higher levels of spasticity, but these studies were done through either chart review or questionnaires (Little et al., 1989). This methodology provides interesting data about how the person perceives their spasticity, but it does not provide objective spasticity measures and therefore may be answering a different question. A recent human study using objective measures found that at chronic time points spasticity was not different across injury severities in human chronic SCI (Sangari and Perez, 2022), which is congruent with our results as well. Another recent human study showed that spasticity varies in persons with anatomically incomplete injuries depending on CST/RST sparing (Sangari et al.,

2019). This is likely not confounding our results as all the incomplete mouse injuries were done on the dorsal side of the spinal cord due to the standard methodology used, but human SCI can originate from any side. One might argue that sparing on the dorsal side might show a preferential development of spasms. Our results that show transection injury, with no sparing, has the same variability of hyperreflexia as the highest functioning incomplete injury group suggest that location may not play a role, though ventral injury comparisons are necessary to confirm.

Overall, these results are instrumental to the understanding of the development of hyperreflexia, translatability of results to other injuries and spinal segments, and the growth of this field.

CHAPTER 2

Introduction

Spasms are involuntary muscle contractions triggered by sensory stimuli and mediated by hyperreflexia to produce strong, multi-joint movements (Levi, Hulting, and Seiger, 1995). These movements can interfere with planned motor function and severely impact the quality of life and safety of individuals. It is unclear what determines the severity of spasms that develop, or whether the mechanism of spasm development is similar across individuals. Understanding the mechanisms underlying the development of spasms would have a profound effect on the treatment of spasms post SCI, the control of movement, and, therefore, the functional independence of people with SCI.

It is known that the serotonin (5HT) signaling system for the spinal cord is intimately involved in contributing to the development of spasms. In the intact spinal cord, the caudal raphe nuclei send descending projections through the dorsal and ventral funiculi to the dorsal and ventral horns of the spinal cord respectively (Xia et al., 2017). The function 5HT serves once it is released in the spinal cord depends on the receptor expression of the postsynaptic cell. Two prominent receptors expressed in the spinal cord are the inhibitory 5HT1 class G-protein coupled receptors (GPCR) and the excitatory 5HT2 class GPCRs (Murray et al., 2011). 5HT1R-expressing neurons have their excitation inhibited by 5HT and are found in sensory circuits in layer III-V of the spinal cord, referred to as the deep dorsal horn (DDH) (Thaweerattanasinp et al., 2020). Neurons expressing 5HT2 class receptors, especially 5HT2AR and 5HT2CR are found throughout the ventral horn (Husch et al., 2012), (Murray et al., 2011). These neurons are made more excitable when exposed to 5HT(Husch et al., 2012),(Murray et al., 2011). Of particular note is how motoneurons are modulated by 5HT. Motoneurons express 5HT2AR and 5HT2CR on their dendrites(Murray et al., 2011). Serotonergic activity in these dendrites leads to the opening of voltage-gated Na+ and Ca++ channels, causing an influx of cations . These persistent inward currents (PICs) lower the threshold for causing the motoneuron to fire an action potential, and increase the firing rate response to suprathreshold input, and prolong the response (Hounsgaard and Kiehn 1985). In addition to 5HT release from the presynaptic neuron and receptor binding, the serotonin transporter (SERT) is involved in 5HT signaling as well. This protein binds free 5HT in the synapse and returns it to the

presynaptic bouton for recycling or degradation. The efficiency of this transporter determines how long 5HT is free to bind to receptors and propagate its effects. Selective serotonin reuptake inhibitors (SSRIs), such as citalopram, inhibit 5HT reupatke and allow more neurotransmitter ligand binding to occur (Shoar, et al., 2021). Overall, 5HT modulates the gain of various spinal circuits, dampening sensory circuits in the DDH, and amplifying the excitability and firing frequency of interneurons and motoneurons in the ventral horn.

Many alterations to 5HT signaling following SCI contribute to the development of spasms. In the deep dorsal horn, interneurons expressing 5HT1B/D/F are disinhibited which unleashes exaggerated input onto their downstream targets (Murray et al., 2011). Ventrally, receptor expression on the motoneurons changes over weeks post injury which reinstates their excitability at 40x baseline (Murray et al., 2010). Interneurons in motor circuits increase their excitatory 5-HT2A receptor expression and become supersensitive to 5-HT (Cao N et al., 2018). Motoneurons increase the proportion of the unedited, constitutively active 5-HT2C receptor isoform (Di Narzo et al. 2014). In summary, several populations increase their input to excitable neurons, causing prolonged firing. These phenomena act in concert to increase the net excitability of the spinal cord and generate spasms. Due to being researched in transection SCI models, it is unknown how residual 5-HT impacts this change in neural modulation. In this study, we are examining how the presence of 5-HT modulates spasm intensity and how that modulation differs with injury severity. The results of this gestalt investigation have implications for identifying populations that are susceptible to modulation of serotonergic targets.

Methods

Spinal cord injuries and mouse grouping- Mice were anesthetized with isofluorane and a laminectomy was performed at the T11 spinal level. In the complete transection group (cTX, n=14), a full transection was performed by cutting the spinal cord with spring scissors. The incomplete impact group (iMP) received incomplete injuries via the Infinite Horizons Spinal Cord Impactor System (IH-0400 Precision Systems and Instrumentation) with a 70kdyne force and 60sec dwell. Using the Basso Mouse Scale (BMS), these mice were scored at 10 weeks post injury and then grouped accordingly as the degree of spared locomotor function has been shown to correlate with tissue damage (Cao et al., 2005), (Scheff et al., 2003), (Schucht

et al., 2002). The groups were delineated as: BMS score ≤ 3 were the incomplete non-weight-bearing (iNWB, n=10) group, BMS score between 3 and 6 were the incomplete weight-bearing (iWB, n=12) group, and BMS score > 6 were the incomplete coordinated stepping (iCO, n=8) group.

Ex vivo sacral cord preparation- This preparation has been previously published (Jiang and Heckman, 2006). Briefly, mice were anesthetized with urethane (.54mg/kg) and the sacral section of the spinal cord was removed. S1-S3 dorsal roots were mounted to stimulating hooks and the ventral roots to recording hooks. The dorsal roots were stimulated at 3x afferent threshold intensity with a 5-train stim with a 10msec interspike interval every minute. Reflex excitability in isolated sacral spinal cords was generally low because of the acute spinal shock. Therefore, low doses of strychnine (1 μM) and picrotoxin (10 μM) were applied to the recording. Electroneurograms (ENGs) of the last 5 responses from the 6 recorded sacral roots were amplified (100×) and band-pass filtered (300–10,000 Hz) before being digitized (20 kHz) for analysis.

Drugs used- 5HT (Sigma-Aldrich) was added to the aCSF in incremental concentrations from 0.1uM to 50uM. The spinal cord was left in each concentration for 1 hour and the last 5 stims were used for analysis. In experiments with citalopram (Sigma-Aldrich), the sacral cord was left in 0.1uM 5HT for one hour before 1uM citalopram was added to the subsequent concentrations of 5HT for one hour to test how SERT inhibition impacted spasm intensity ininjuries of varying severities.

Spasm epochs- To quantify the spasm intensity, the integral over several time points of the rectified ENG was calculated. These epochs correspond to motoneuronal firing due to different inputs as defined previously (Murray et al., 2011). The signal was rectified and integrated over 1 and 10 seconds of LLR (LLR1, LLR10). These two time points were chosen to both examine the highest intensity of LLR(LLR1) and have a measure that corresponded to duration (LLR10) (average responses were 8 seconds long *ex vivo*). Reflex magnitude was calculated as rectified, integrated ENG (riENG). 5HT responses were normalized to baseline responses such that:

normalized response=(5HTresponse-baseline response)/baseline response.

Statistical Analysis- This work was exploratory in nature, rather than confirmatory, and therefore no formal power analysis was conducted. Density plots were generated to explore the distribution of outcomes of

interest. Side-by-side boxplots were generated across injury group and channel to explore the difference between groups. Generalized linear mixed effect models were fit on the normalized responses with a random intercept for mouse and stimulation within mouse as well as fixed effects for injury group, 5HT concentration, and the interaction term between injury group and 5HT concentration. ANOVA was conducted to test whether the interaction term is statistically significant.

The correlation of outcomes of interest between *in vivo* and *ex vivo* data was explored through generalized linear mixed effect models with a random intercept for mouse and stimulation within mouse as well as fixed effects for which dataset the outcome originates, and injury group. ANOVA were conducted to test whether the injury group significantly improves the model fit. Average values of outcomes were calculated for each mouse across channels.

The amount of positive and negative normalized responses between injury groups was tested with Chi squared analysis to test whether there were differences in the proportion of amplification or suppression between the injury groups.

Results

Effect of injury severity on 5-HT modulation of ex vivo spasms

Serotonergic modulation of *ex vivo* hyperreflexia was measured by adding increasing concentrations of 5HT to the *ex vivo* sacral cord preparation. Figure 2.1 shows the overall ventral root response to different concentrations of serotonin in 3 epochs post spasm, LPR, LLR1, LL10. The LPR epoch shows motor neuron response to polysynaptic input to the motor neuron. The LLR1 response shows the first second of PIC activation. The LLR10 response shows 10 seconds of the PIC activation which incorporates both the amplification and the prolongation of the PIC.

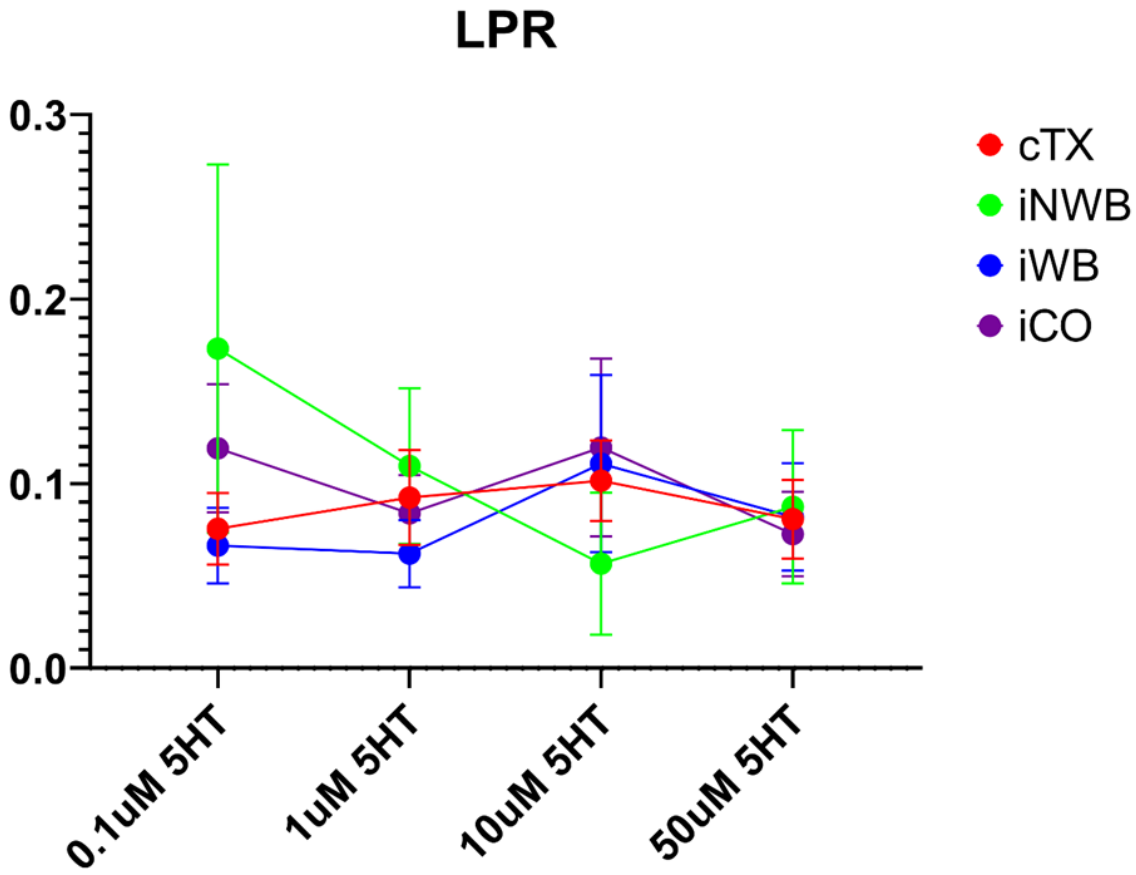

Figure 2.1 Increasing serotonin concentration does not have a significant effect on the LPR (F=0.317, p=0.813) and the injury groups are not significantly different from one another (F=0.105, p=0.957). Each line represents the area under the rectified curve of the LPR at the appropriate 5HT concentration normalized to the area under the rectified curve of the LPR without 5HT of a different injury group. Error bars display standard error of the mean.

In the LLR10, but not the LPR or LLR1, increasing concentrations of 5HT significantly increase the excitability of the ventral root response. Therefore, serotonin has an excitatory effect on motoneuron excitability via the PIC.

Increasing concentrations of 5HT had a significant increase in the spasm intensity in the LLR10 (F=5.531, p=0.001). Post hoc analysis revealed significant differences were between the 0.1uM trial and the 10uM and 50uM trials in (0.1uMx10uM| p=0.012, 0.1uMx50uM| p=0.006).

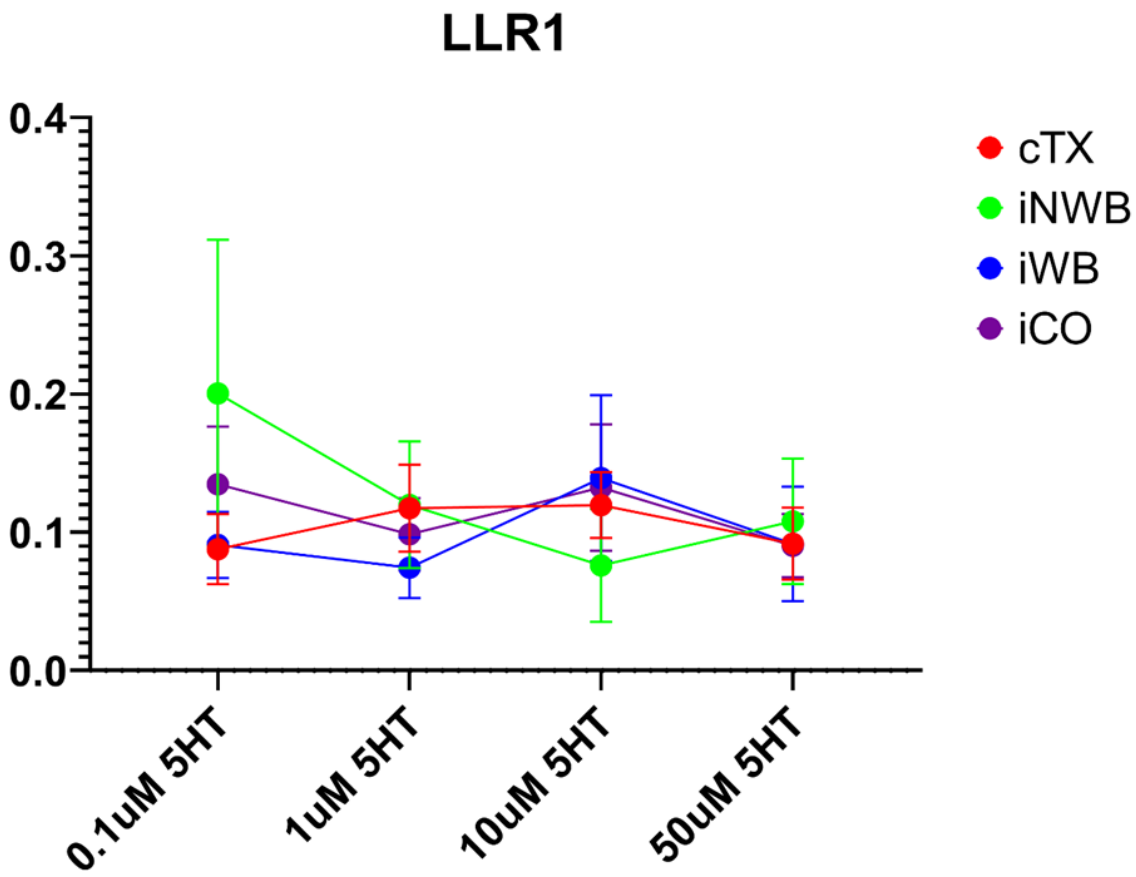

Figure 2.2 Increasing serotonin concentration does not influence the LLR1 (F=0.356, p=0.785) and the injury groups are not significantly different from one another (F=0.062, p=0.98). Each line represents the area under the rectified curve of the LLR1 at the appropriate 5HT concentration normalized to the area under the rectified curve of the LLR1 without 5HT of a different injury group. Error bars display standard error of the mean.

Differences between the functional groups also became apparent with the addition of 5HT ((*F*=3.705, *p*=0.016). In the LLR10, our measure of sustained spasms, the iNWB group on average had a greater increase in response to 5HT than the other three groups ((iNWBxcTX| *p*<0.001, iNWBxiWB| *p*=0.010, iNWBxiCO| *p*<0.001)).

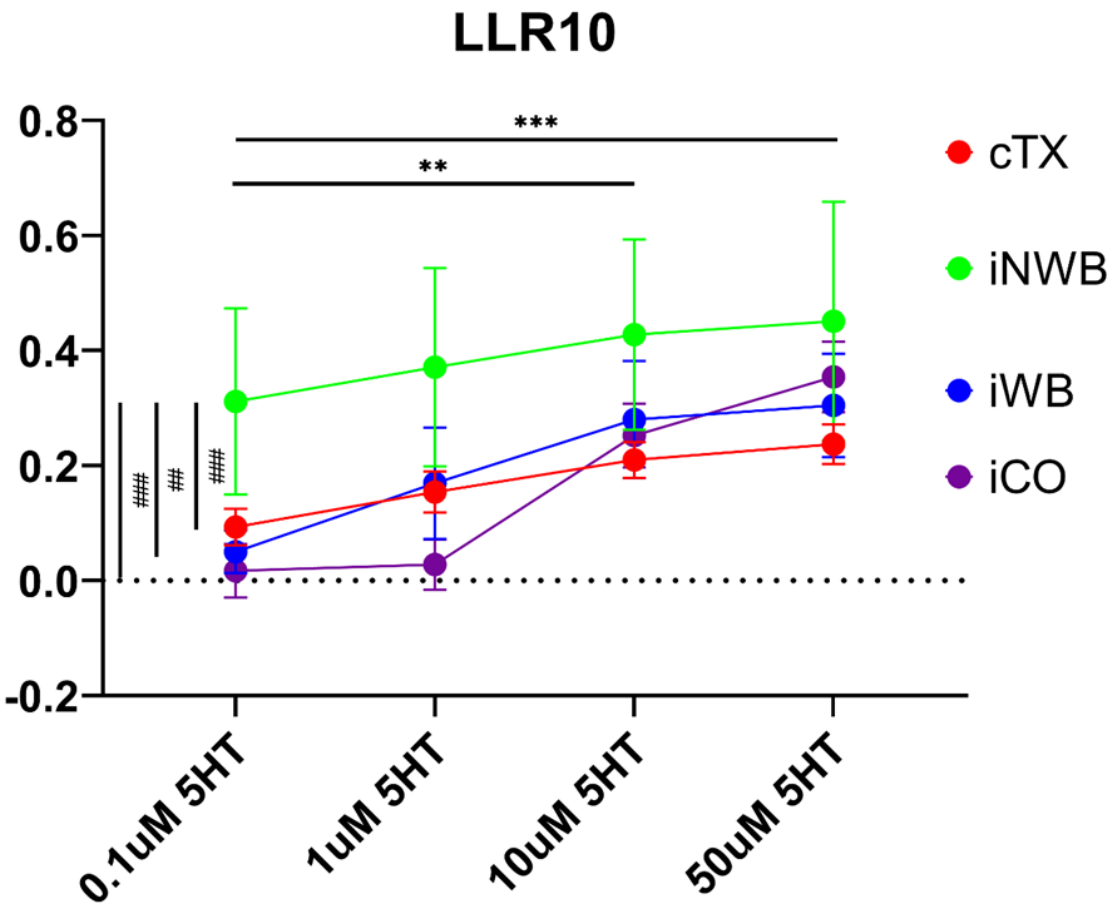

Figure 2.3 The amplification of LLR10 spasm intensity by 5HT is more robust in higher concentrations of 5HT and in severe impact injuries. in the long LLR measure, both the serotonin concentration (F=3.705, p=0.016) and the injury group (F=5.531, p=0.001) have a significant effect on the % change in spasm intensity. In particular, greater concentrations of serotonin increase the amplification compared to the lowest concentration of serotonin(0.1uMx10uM| p=0.012, 0.1uMx50uM| p=0.006) and the non-weight bearing group has a significantly greater amplification of spasm intensity compared to the other three injury groups(iNWBxcTX| p<0.001, iNWBxiWB| p=0.010, iNWBxiCO| p<0.001). This data suggests that spasms in more severe, but incomplete SCI can be modulated to a greater extent compared to other injury severities by serotonin, particularly in how serotonin signaling interacts with the motoneurons that sustain spasms. Each line represents the area under the rectified curve of the LLR10 at the appropriate 5HT concentration normalized to the area under the rectified curve of the LLR10 without 5HT of a different injury group. Error bars display standard error of the mean.

Ventral root suppression by 5-HT

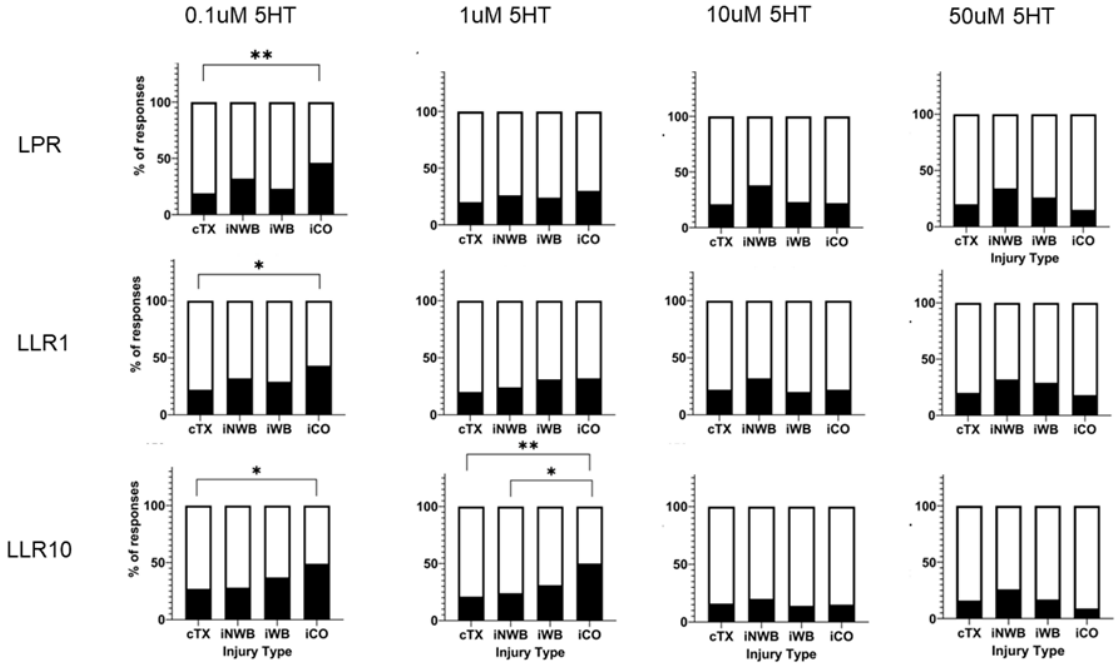

Figure 2.4 Overall excitatory response in part due to increase in switch from inhibitory response to excitatory response to 5HT At 0.1 uM 5HT, there is a significant difference in the proportion of roots that are excited by 5HT or inhibited by 5HT in the cTX vs iCO groups in all 3 epochs (A, B, C) (LPR: p=0.054, LLR1: p=0.0433, LLR10: p=0.0281). In LLR10, this difference persists in 1uM 5HT as well. (iCOxcTX p=0.0013, iCOxcTX p=0.0105)

Although the central tendency was for 5HT to increase the spasm intensity, there were instances where adding serotonin decreased the spasm intensity. Tallying the number of positive and negative responses in each injury group revealed that the iCO group has a significantly larger portion of suppressed responses in the LPR, LLR1, and LLR10 than cTX in the presence of 0.1uM of serotonin (LPR: p=0.054, LLR1: p=0.0433, LLR10: p=0.0281). The difference in responses disappears in greater concentrations of 5-HT. In the LLR10 the iCO group had a significantly smaller proportion of positive responses compared to both the cTX group (p=0.0013) and the iNWB group (p=0.0105) in 1uM 5HT.

5-HT reuptake

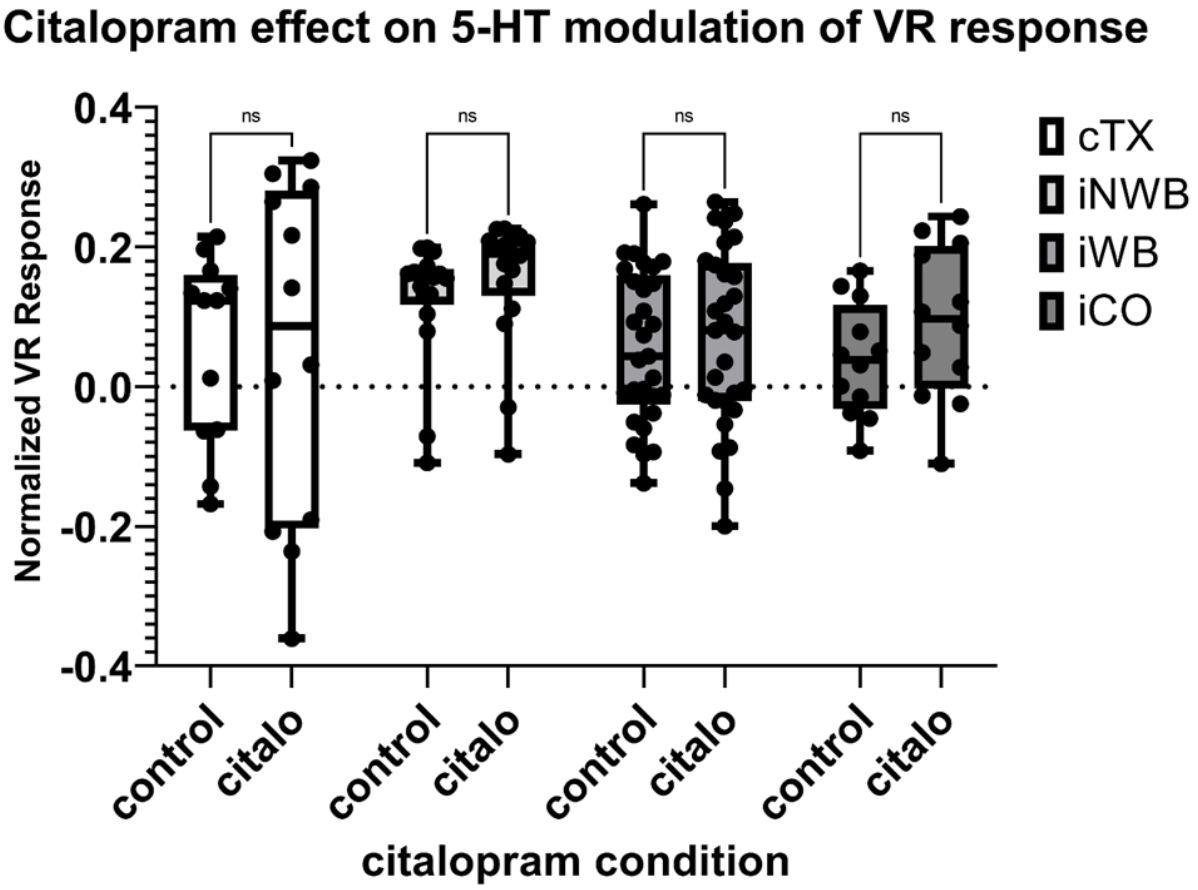

Figure 2.5 Serotonin reuptake does not change post chronic SCI

Blocking serotonin reuptake did not change ventral root response across injury severities.

The 5HT reuptake system was tested by adding the SSRI citalopram to the ex vivo preparation in the presence of 0.1uM 5HT. Citalopram did not have a significant effect on the ventral root output of the spinal cords.

Discussion

It is interesting that there were no differences in raw values, but there were in the normalized increase with 5HT, suggesting that despite the variability in spasm intensity within injury groups, the degree that the spasms are modulated by 5HT is different.

The main effect of 5HT concentration shows that spinal cord output maintains sensitivity to 5-HT. This was true across injury severities so none of the groups completely lost their sensitivity to 5HT. This means that despite the spinal cord developing alternative forms of excitability, the sensitivity to serotonin is maintained, however to what extent seems to depend on the type of injury received.

In individuals that received more severe impact injuries we find a consistently larger increase with the addition of 5HT. The iNWB group has a greater % change compared to the other injury groups in the LLR10 measure. In LLR10 the iNWB group had a greater % change than the other group. The difference seems to suggest that a more severe injury will lead to greater enhancement of spasm intensity by 5HT as long as there are still spared fibers. It is interesting that the iNWB group has a significantly stronger LLR10 than even the fully transected cTX group, suggesting that motoneuron excitability is enhanced somehow by spared fibers. Looking at the individual root data suggests that there is a subset of super spasms in the iNWB group. Further analysis is necessary to determine what causes this subset to occur. One possible explanation is full spinal plasticity combined with some amount of spared fibers.

The positive/negative results show that there were some responses that were lessened by 5HT. This phenomenon could be explained in three ways: (1) inhibition of spasm trigger (2) enhancement of motoneuron inhibition, and (3) suppression of motoneuron excitation via 5-HT. The first possibility is mediated by 5HT1Rs on DDH neurons in sensory circuits (Thaweerattanasinp et al., 2016). These species of neuron constitute the zolmitriptan-sensitive neurons whose pharmacological modulation via 5HT1B/D/F receptors lessen the firing of motoneurons during the LPR phase (Murray et al., 2011). Depending on the receptor expression in these particular neuron species, exposure to serotonin will limit their firing and thus weaken the trigger, resulting in a weaker spasm. A lessening of the trigger would manifest as differences in the LPR, LLR1, and LLR10 thus triggering a less intense spasm. Exposure to 5HT can also enhance the inhibitory circuits to motoneurons. Inhibition is very potent for the cessation of PICs (Hounsgaard et al., 1984). Changes here would manifest as differences in LLR10 without differences in LLR1 or LPR as the spasm is being cut short by the inhibition. In our preparation, however,

we used a picrotoxin and strychnine mix to increase spinal cord excitability, so while this source of variability may exist in natural SCI cases, differences would need to be assessed in the absence of picrotoxin and strychnine. Although 5-HT has been shown to enhance PICs and enhance motor output (Hounsgaard et al., 1984), in certain preparations it has been shown that 5HT lessens motor output and contributes to fatigue. In the turtle spinal cord preparation it was shown motoneurons have a dose-dependent response to 5HT such that lower concentrations of 5HT enhance motoneuron firing through 5HT2ARs and 5HT2CRs, while higher concentrations flood over into 5HT1ARs proximal to the axon hillock and inhibit firing (Perrier and Cotel, 2008). Differences due to motoneurons inhibition by 5HT would manifest as less intense LPR, LLR1, and LLR10. While motoneuron amplification has been shown to become 40x more sensitive to 5HT following SCI, it is unclear how motoneuron inhibition sensitivity is modulated by SCI. Although we did not observe a suppression of ventral root output in higher concentrations of 5HT, further research into these differences would look for depression of the dose response curve of motoneuron firing rate amplitude at higher (>50uM) concentrations of 5HT. Individuals with inhibited motoneuron fatigue would be expected to be less susceptible to fatigue when performing rigorous activity as well as more spasms triggered by this physical activity, as the 5HT1 system is no longer inhibiting motoneuron output at high points of arousal and thus monoaminergic activation.

The ratio of excitation and inhibition is more even in the iCO group as evidenced by the greater proportion of negative responses at the lowest concentration of 5HT. This could also tie into why the enhanced modulation of the change in output observed in the iCO mice compared to cTX mice occurred. By disrupting the balance of excitation and inhibition with serotonin, without increasing the motoneuron's excitability or the amount of input to the motoneuron. Parsing apart what mechanism would require intracellular recordings from motoneurons to assess differences in the electrical properties, EPSP and IPSP amplitudes in the motoneurons receiving different severities of injury.. Although we controlled the concentration of in the aCSF filtered through the system, we could not control how the concentration of 5HT was diffused throughout the spinal cord and thus we cannot be sure the precise concentration of 5HT at a particular point in the spinal cord and how this compares to what the circuits see *in vivo*.

The lack of difference with citalopram means that we have not demonstrated differences in 5HT reuptake mechanisms following spinal cord injury. If the citalopram had had an effect, there would have

been a significant increase in the ventral root output with the addition of citalopram. It is possible that the effect of 5HT reached a ceiling and thus did not potentiate with the addition of citalopram.

Altogether these results suggest that the degree to which 5HT modulates spasm intensity varies depending on injury severity, with more severe impact SCI having a greater increase in ventral root output in the presence of 5HT and less severe injuries being more moderately modulated. This may be due to a shift in excitatory and inhibitory effects of 5HT that depend on injury severity where less severe injuries have a more even balance between positive and negative responses at lower concentrations of 5HT. This may be indicative of a severely injured subpopulation that would receive significant therapeutic benefit from targeting the 5HT system to ameliorate spasms.

DISCUSSION

A. INTERPRETATION OF RESULTS

A.1 Similar range of spasm intensities across injury types and functional outcomes

One explanation could be that mechanisms underlying hyperreflexia are weighted more toward spinal changes as adding spared fibers in the *in vivo* approach did not change the overall result. If this is the case, while the overall intensity of spasms may be similarly variable across injury severities, individuals with more spared fibers may be more susceptible to having spasms triggered by volitional movement as their spared descending input is now synapsing into a system with greater net excitability. We only elicited spasms with electrical stimulation rather than observing spontaneous spasms the animals experienced in day-to-day activities. One research strategy our lab has been looking into is using MoSeq to identify spasm behavior. This behavioral testing technique observes the free roaming mouse with infrared tracking and uses machine learning algorithms to identify stereotyped primitive motor syllables that make the building blocks of all of a mouse's motor behavior (Datta, 2019). This technique could be used on mice with SCI to identify the roster of motor primitives in the injured mouse and presumably spasm behavior could be extracted and quantified. If spared fibers serve to increase the amount triggers for spasms without affecting the intensity one would expect higher spasm frequency and more spasms behavior following volitional behaviors that are not present in mice with less motor function.

In our studies we used the established correlation between functional outcome and injury severity to determine the damage done by our impact injuries. It would be illuminating to perform histology on the spinal cords of injured animals to ascertain the extent of sparing following each injury. Not only is the amount of spared white matter important to make note of, but also the location of the sparing as well, as we could glean an understanding of what tracts, if any, are important for the prevalence of spasms. To this end, using alternative forms of injury to damage other portions of the spinal cord preferentially would also be informative for identifying critical areas for the development of spasms. In our study we consistently used dorsal impact injuries and this will preferentially damage the dorsal tracts and

innervation to the DDH. There are tracts that when damaged come with greater functional loss compared to others. Fouad looked at dorsal vs ventral sparing in mice and found no difference between the two injuries, but it is unknown how this will play out with spasm intensity as the outcome measure compared to motor function(Schucht et al., 2002) . Perez's group found the mediolateral tracts correlated with spasticity in humans, it is unknown if spasms in mice if this is the case (Sangari et al., 2019).

The development of hyperreflexia may be dependent on an on/off mechanism triggered by SCI, but not modulated by the degree of injury, such as a trigger in an inflammatory pathway. ADAR2 has been found to negatively correlate with inflammatory genes via RNASeq. ADAR2 is involved in regulating editing of 5HT2C isoforms (Di Narzo et al., 2015). This evidence suggests that neuroinflammatory interactions are responsible in part for spasm generation. Neuroinflammation is heavily disrupted by SCI. The blood brain barrier is compromised causing infiltration of circulating immune cells (Arevalo-Martin et al., 2016). While the inflammatory response usually dies down within several weeks, there are cases of chronic proinflammatory tone (Allison and Ditor, 2014). One strategy to investigate this would be to look for identifying genes of certain inflammatory cell types and see which of those correlates with more intense spasms. One challenge is ascertaining when the switch is triggered. If it is triggered during the acute phase of injury, immunotherapy during the acute phase of recovery would be effective in changing whether someone is likely to develop spasms, but not so good at treating the symptoms once they begin as all of the inflammatory signaling you are trying to target will be gone. If this ADAR2 pathway is something that is maintained by chronic inflammation, however, targeting the immune system may prove effective after treating long enough for turnover with 5HT2c expression.

If immune modulation is effective, a cannabinoid strategy may be useful in modulating spasms. The cannabinoid receptor CB2R is expressed on many types of immune cells and it promotes an anti-inflammatory tone, potentially counteracting the neuroinflammatory signaling that initiates the development of spasms (Arevalo-Martin, et al., 2014).

Another thing to consider is whether targeting problematic cell types that correlates with more intense spasms, or deducing the signaling pathway that contributes to 5HT2C editing is a better treatment strategy. When targeting the immune cells, some off-target effects may be negating beneficial effects of the immune cell's presence. When it comes to discerning the signaling pathway, It would help to know

what immune cell species to search for so that you have some understanding of the signaling paths to target. One difficulty in this will be tissue management. In order to record the full spinal network activity you would need to use the sacral portion of the spinal cord to record from and spinal cord rostral from there to harvest for immune cell identification. Alternatively, in vivo spasms could be used as the final measure.

An alternative explanation for this result could be that the various contributing neuronal groups may have different spinal neuronal excitation changes post injury, resulting in variable hyperreflexia. While it is known that 5HT1B/D/FR-expressing interneuron populations become disinhibited and motoneurons fall silent during acute spinal shock and then regain their excitation through 5HT2C receptor expression changes (Murray et al., 2011), it is unclear how populations beyond these two behave following SCI and how their changes impact spasms. It is already known that two different neuron species have their excitability modulated in opposite directions following injury, it is unclear how other populations behave. Adding to the fog is the contribution of a particular neuron population's change in excitation following injury and finally how different amounts of sparing affect that contribution.

There are ways to control these factors depending on what question you want to ask. One way to account for sparing is lateral hemisection or impact injury and comparing spasms elicited on the injured side to spasms on the uninjured side. Tracts that cross over would not be accounted for, but the tracts that remain ipsilateral can be considered to have been removed from the injured side. Genetic labeling would be effective in testing the effects of particular cell types. Optic stimulation of the sacral cord has been shown to be effective to modulate V3 interneuron firing (Lin S et al., 2019), so it may be possible to genetically tag and optically stimulate other neuron populations. Using retrograde viruses, interneurons that synapse directly onto motoneurons can be genetically labeled (Zhang et al., 2017). These neurons can then be harvested for genetic identification, or recorded from to ascertain their validity as a spasm trigger. A difficulty that occurs when trying to both record and genetically identify cells in the complete preparation is that the cells that are being recorded are several layers of neural tissue deep, making it difficult to record from these cells with an electrode that can also denucleate the cell. Dye filling and then looking for the recorded cell in the sacral cord preparation is like finding a needle in a haystack. The benefit to the sacral cord preparation is that it keeps the extensive arborization of the motoneuron

dendrites intact and preserves the prolonged spasm firing behavior, but the amount of tissue used in the preparation makes certain techniques difficult. The ability to elicit spasm behavior in a slice preparation would make pharmacological and genetic studies more amenable and behavior of discrete populations following injury easier to isolate.

Our results were a peek into the overall ventral root output to see if it was different. A deeper dive into what mechanisms account for the variability may provide a clearer picture into which changes relate to which IMB phenotypes. Our results that show transection injury, with no sparing, has the same variability of hyperreflexia as the highest functioning incomplete injury group suggest that location may not play a role, though ventral injury comparisons are necessary to confirm. SInce our injuries were dorsal impact injuries, the dorsal spinal cord was preferentially injured. In terms of 5HT tracts that would mean the 5HT supply to the dorsal horn is preferentially damaged, leading to more disinhibition of sensory circuits compared to if the injuries came from any side. If we were to compare these dorsal injuries with more ventral injuries, we might expect more intense LLRs compared to LPR, as there would be less damage to the 5HT supply to the DDH, keeping those neuron populations inhibited and ameliorating the trigger while the ventral horn undergoes plasticity and becomes more excitable.

A.2 Difference in amplification of spasms by 5HT

The story becomes more complicated with modulation by 5HT. Although we found that the intensity of the spasms elicited by the injury groups were not different, the amount that their ventral root output is modulated by 5HT is different. The iNWB group ventral root output was more sensitive than the other impact groups. It appears that there is a group of super spasm mice that is not seen in the other injury groups. One possibility is that certain neuron populations are supersensitive to 5HT and contribute more to spasms in these animals. The V2a population could be contributing heavily to spasms in these mice.Since there is a population of V2a interneurons that synapses directly onto motoneurons, they could make a potent spasm trigger in the presence of residual 5HT.

The ratio of excitation and inhibition is more even in the iCO group as evidenced by the greater proportion of negative responses at the lowest concentration of 5HT. This could also tie into why the enhanced modulation of the change in output observed in the iCO mice compared to cTX mice occurred.

By disrupting the balance of excitation and inhibition with serotonin, without increasing the motoneuron's excitability or the amount of input to the motoneuron. Parsing apart what mechanism would require intracellular recordings from motoneurons to assess differences in the electrical properties, EPSP and IPSP amplitudes in the motoneurons receiving different severities of injury. Although we controlled the concentration of in the aCSF filtered through the system, we could not control how the concentration of 5HT was diffused throughout the spinal cord and thus we cannot be sure the precise concentration of 5HT at a particular point in the spinal cord and how this compares to what the circuits see *in vivo*.

B. FUTURE DIRECTIONS

B.1 Investigating inflammation

An inflammatory pathway may be an on/off mechanism for spasms that is not modulated by injury severity. Inflammatory genes negatively correlate with ADAR2 via RNASeq. 5HT2C isoform editing is regulated in part by ADAR2 (Di Narzo et al., 2015). Neuroinflammatory interactions could therefore contribute to spasm generation. Neuroinflammation is heavily disrupted by SCI. Not only does the spinal cord receive direct damage from its noxious insult, but the following inflammatory response exacerbates and radiates the damage to the spinal cord. The blood brain barrier is also comprimised. While the inflammatory response usually dies in weeks, there are cases of chronic proinflammatory tone. One strategy to investigate the neuroinflammatory interactions that beget spasms would be to look for identifying genes of certain inflammatory cell types and see which of those correlates with more intense spasms. One challenge is ascertaining when the switch is triggered. If it is triggered during the acute phase of injury, immunotherapy during the immunotherapy would be effective in changing whether someone is likely to develop spasms, but not so good at treating the symptoms once they begin as all of the inflammatory signaling you are trying to target will be gone. If this ADAR2 pathway is something that is maintained by chronic inflammation, however, targeting the immune system may prove effective after treating long enough for turnover with 5HT2c expression.

If immune modulation is effective, a cannabinoid strategy may be useful in modulating spasms. The cannabinoid receptor CB2R is expressed on many types of immune cells and it promotes an anti-inflammatory tone, potentially counteracting the neuroinflammatory signaling that initiates the development of spasms (Arevalo-Martin et al., 2016).

B.2 Dissecting populations and circuits

While we were able to detect differences in the net excitability in the spinal cords, it is important now to understand what neuron populations were interacting with 5HT to manifest the differences we observed. One line of questioning would involve seeing how specific neuron populations' 5HT signaling change in different injury severities. Another line of questioning utilizes pharmacological strategies to target particular receptors. By increasing the knowledge of how sparing and injury severity impact 5HT signaling we can develop a comprehensive picture for how spasms emerge from a multitude of alterations in the spinal cord network. These mechanisms could potentially be altering independently of one another in each mouse thus creating the aggressive variability that we see in our injury and functional groups. Rather than injury severity, the subpopulations amongst spasms could be due to the dominance of changes in certain neuronal populations. The subgroup of high responders could potentially have their spasms driven by V2a neurons with heightened 5HT sensitivity. Another subpopulation of spasms could be driven by V3 excitation.

B.3 How do these results compare to humans?

When comparing the functional groups in our studies to human SCI, there is a general agreement in the results. First, it is notable that our rodent spasm data is very variable much like human spasticity. One point of interest was the initial apparent disagreement between Perez's human studies on spasticity and our mouse studies. Perez found that individuals with motor incomplete injuries and thus spared fibers had more intense spasms than those with complete injuries, while we found no difference between our cTX group and the impact injuries. Perez's later studies went on to find that amongst those with motor incomplete injuries, those with more RST sparing than CST sparing had more intense spasms. Because her subject received lesions from all around the spinal cord, she was able to find variance in the

CST/RST ratios. Our dorsal impact injuries rested right above the dorsal CST and thus our mice would presumably all have very low CST/RST ratios so that is one source of variability that our injury model lacks. Another finding Perez made in her 2022 paper is that differences in spasticity that are present at subchronic timepoints receded at a later timepoint. Our mice could be following a similar trajectory and we sampled them at a very chronic timepoint when the differences between injury severites could have washed out as well.

Another factor to take into account is the difference in dependence on descending input between rodent motor function and human motor function. Rodents are able to recover much more robustly compared to humans. If a human spinal cord loses descending input, it becomes much more difficult to recover motor function than for a rodent spinal cord, suggesting that spinal circuitry is more sufficient to execute motor output in the rodent and thus the effects of spared fibers in the mouse could be more modest due to the difference in contribution of descending input between the two species (Hanna, et al., 2019).

These studies reveal the need to better understand the mechanisms underlying spasms to be able to more effectively classify and treat them. Despite varying the damage to functional recovery, there remained much variability within the functional groups. The subgroup of severely injured mice that seems to have spasms super sensitive to serotonin begins to tug at the possibility that spasms vary in the mechanisms that drive their intensity and further pursuits should try to categorize by what factor different groups of spasms are modulated by in order to develop therapies that precisely and effectively ameliorate the intensity and frequency of spasms and thus ease the burden of living with a SCI.

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