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TITLE: Spinal Cord Injury-Induced Dysautonomia via Plasticity in Paravertebral Sympathetic Postganglionic

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<p><b>ABSTRACT</b> Sympathetic <u>postganglionic</u> neurons (SPNs) located in sympathetic ganglia represent the final common sympathetic motor output. Even though SCI produces a profound plasticity in sympathetic autonomic function, the extent that SCI-induced dysautonomia is based on SPN changes within the thoracic paravertebral sympathetic chain is unknown. Given their strategic site in autonomic signaling to body, any plasticity is likely to be of high significance, yet there is a paucity of studies undoubtedly due to their near anatomical inaccessibility. We have solved the accessibility problem with a strategic methodological advance. We will determine the extent to which paravertebral SPNs are a nodal site for vasomotor dysfunction after SCI.</p> <p>We will undertake physiological, pharmacological and optogenetic studies to examine network and cellular plasticity induced by SCI to answer the following two questions: (a) Does SCI lead to plasticity in synaptic interactions between preganglionics, SPNs and primary afferents? (b) Do SPNs become hyperresponsive to synaptic inputs after SCI?</p>					
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## 1. INTRODUCTION:

Sympathetic postganglionic neurons (**SPNs**) located in sympathetic ganglia represent the final common sympathetic motor output. Even though SCI produces a profound plasticity in sympathetic autonomic function, the extent that SCI-induced dysautonomia is based on SPN changes within the thoracic paravertebral sympathetic chain is unknown. Given their strategic site in autonomic signaling to body, any plasticity is likely to be of high significance, yet there is a paucity of studies undoubtedly due to their near anatomical inaccessibility. We have solved the accessibility problem with a strategic methodological advance. We will determine the extent to which paravertebral SPNs are a nodal site for vasomotor dysfunction after SCI.

We will undertake physiological, pharmacological and optogenetic studies to examine network and cellular plasticity induced by SCI to answer the following two questions: (a) Does SCI lead to plasticity in synaptic interactions between preganglionics, SPNs and primary afferents? (b) Do SPNs become hyperresponsive to synaptic inputs after SCI?

## 2. KEYWORDS:

*spinal cord injury, sympathetic, autonomic, autonomic dysreflexia, spinal cord, electrophysiology, plasticity, paravertebral, postganglionic*

### 3. ACCOMPLISHMENTS:

*The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

- a. What were the major goals of the project?
1. List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

<b>Characterizing thoracic chain sympathetic postganglionics</b>		
<b>Major Task 1a: Convergence and divergence</b>	<b>months</b>	<b>% completion/ Completion dates</b>
Subtask 1: Segment specific properties	1-6	75%
Subtask 2: Pharmacology	7-12	50%
Subtask 3: Breeding/crossing transgenic mice and spinalizations	1-36	3months behind target
Subtask 3: Establish intracellular recording techniques	3-18	100%
<b>Major Task 1b: Convergence and divergence</b>	<b>months</b>	
Subtask 1: Incorporation of optogenetic approaches for selective activation of neuron populations	12-18	25%
<b><u>Milestone(s) Achieved:</u> Understanding of synaptic organization in uninjured mice and ability to use optogenetics to selectively activate afferent and efferent fiber populations</b>		
<b>Intracellular recordings and optogenetics</b>		
<b>Major Task 2: Characterize mechanisms responsible for dysautonomia after spinal cord injury using intracellular recordings and optogenetics</b>	<b>months</b>	<b>% completion/ Completion dates</b>
Subtask 1: Physiological plasticity in preganglionic-postganglionic interactions assessed using optogenetics	18-36	0%
Subtask 2: Physiological plasticity in afferent-postganglionic interactions assessed using optogenetics	18-36	0%
Subtask 3: Physiological plasticity in preganglionic-afferent interactions assessed using optogenetics	18-36	0%
Subtask 4: Intracellular recordings of synaptic and cellular plasticity in membrane properties; demonstration of membrane bistability	18-36	5%
<b><u>Milestone(s) Achieved:</u> Demonstration of important contribution of thoracic sympathetic chain to SCI-induced autonomic plasticity and forward insight into therapeutic interventions for future study</b>		
<b>Data analysis and publications</b>		
<b>Major Task 3: Data analysis and publications</b>	<b>months</b>	<b>% completion/ Completion dates</b>
Subtask 1: Data analysis	6-36	25%
Subtask 2: Manuscript writing and submission	24-36	10%
<b><u>Milestone(s) Achieved:</u> Dissemination of scientific results.</b>		

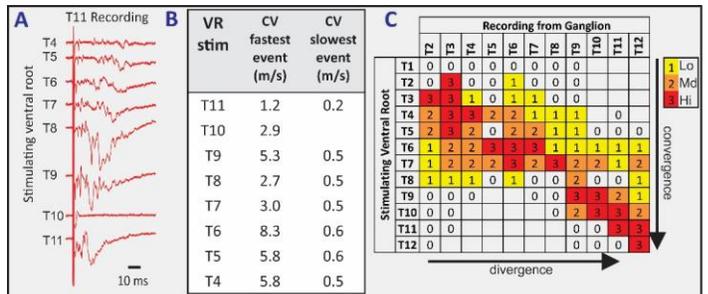
b. What was accomplished under these goals?

1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative);

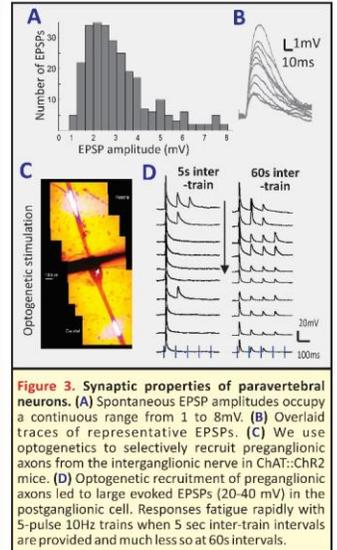
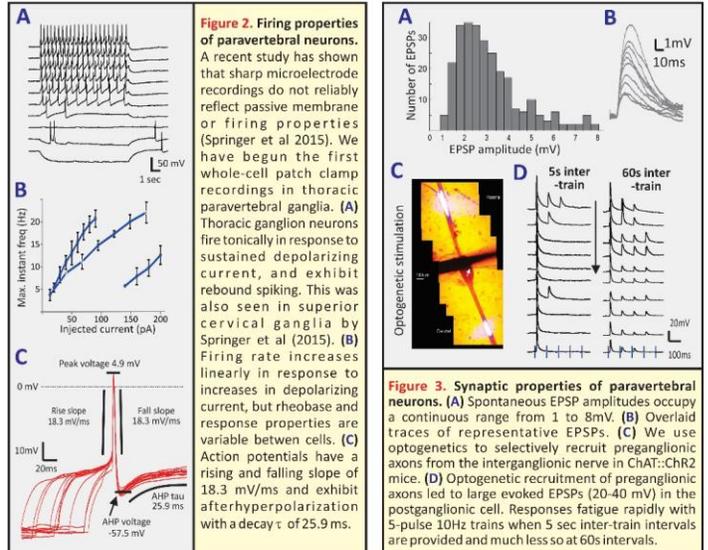
**Convergence and divergence of inputs in thoracic chain ganglia.** [Fig 1] Properties of preganglionic divergence and convergence onto thoracic chain SPNs was previously examined in guinea pig<sup>6,7</sup>. We began to undertake similar studies in the adult mouse and observed comparable patterns of convergence and divergence. Moreover recruited of presynaptic events covered a comparable range of conduction velocities<sup>6</sup>.

**Characterization of cellular properties in adult mouse thoracic paravertebral ganglia.** [Fig 2] A major function of sympathetic paravertebral chain ganglia neurons is to maintain vasomotor tone. While the functional properties of cervical and lumbosacral paravertebral ganglia neurons have been characterized, little is known about the functional properties of neurons within thoracic paravertebral ganglia.

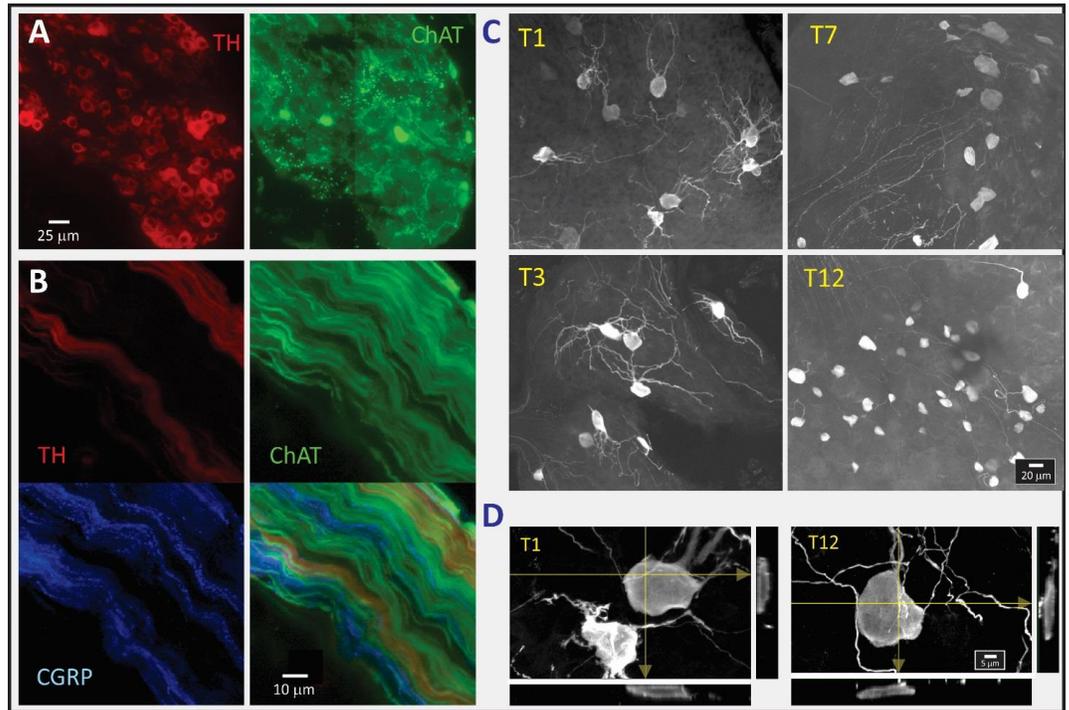
We developed an approach that allows for whole-cell patch clamp recordings in intact thoracic ganglia to characterize cellular and synaptic properties. We recorded from 8 cells deemed of good quality and obtained the following mean values  $\pm$  SD: resting membrane potential ( $-66 \pm 10$  mV), membrane resistance ( $526 \pm 235$  M $\Omega$ ),  $\tau_m$  ( $54 \pm 25$  ms), and rheobase ( $76 \pm 47$  pA). Threshold voltage was typically 10 mV higher than resting membrane potential, action potentials displayed after-hyperpolarization and some cells displayed post-inhibitory rebound. All neurons were capable of repetitive firing. Maximal firing rates observed in response to depolarizing current steps ranged from 14-17 spikes/sec. During intracellular depolarization, firing rate increases with increased current injection and cells sustain tonic firing. Spike frequency adaptation was also observed. All recorded properties are fully consistent with those reported recently with whole cell recordings in rat superior cervical ganglia. Strikingly, our recorded properties differ substantially from sharp electrode recordings obtained from adult mouse tSPNs. Our recorded membrane resistance is 4.5 fold higher and  $\tau_m$  is 7.5 fold longer than observed by Jobling and Gibbins (1999), and our neurons only fired tonically (e.g. Fig 4) while theirs only fired phasically to depolarizing current pulses. Note that all of these differences are consistent with greater cell damage caused by sharp electrode penetration compared to patch clamp recordings. We therefore assume that our new whole cell recordings are closer to physiological reality.



**Figure 1.** Convergence and divergence of inputs onto thoracic chain ganglia. (A) Example of convergent input onto the T11 ganglion. (B) Conduction velocities range between 0.2-8.3 m/s - comparable to that observed in guinea pig (Blackman & Purves, 1969). (C) Map of convergence and divergence in another animal. Estimates of preganglionic convergence onto individual ganglia are shown as columns progressing from T1-T12 color-coded by projection magnitude. Preganglionic divergence is shown in rows. Patterns are comparable to guinea pig (Lichtman et al 1980).



Patch clamp recordings in combination with optogenetics in another cell from a ChAT-ChR2 mouse suggested that postganglionic neurons receive massive cholinergic input from preganglionic neurons. Repetitive (10 Hz) optical stimulation of cholinergic preganglionic axons elicited a large EPSP in the postganglionic neuron (20 mV). The EPSP was usually suprathreshold and accompanied by an action potential. The response fatigued dramatically to repetitive stimuli. This seems to indicate that presynaptic release of acetylcholine attenuates rapidly on the order of 100-300 ms. Together, these data suggest that presynaptic release of acetylcholine by preganglionic neurons is the rate-limiting step for transmission in thoracic paravertebral ganglia.

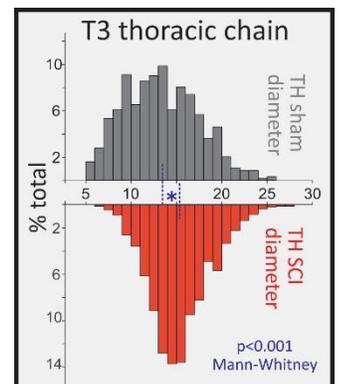


**Figure 4. Neurochemistry and anatomy of thoracic chain ganglia.** (A) Most cells are adrenergic and identified by immunolabeling for tyrosine hydroxylase (TH), while a minority (3-5%) are cholinergic, identified by their expression of choline acetyltransferase (ChAT). (B) Axons within interganglionic nerves are TH+ postganglionic adrenergic, ChAT+ preganglionic, and CGRP+ visceral afferents. (C) Morphology of individual postganglionic neurons in several thoracic chain ganglia revealed by TH-Cre line with sparse labeling. While TH::tdTomato neurons from rostral thoracic chain ganglia contain dendrites (T1 and T3 are shown at left), more caudal thoracic ganglia lack clear dendritic arborizations (T7 and T12 are shown at right). Overall, TH neurons from T4-T12 ganglia did not contain observable dendrites. (D) TH::tdTomato labelled adrenergic neurons with putative somatic adrenergic synapses.

**Synaptic properties of paravertebral neurons.** [Fig 3] Spontaneous EPSP amplitudes occupy a continuous range. For the neuron shown, this was from 1-8mV. Using (ChAT)::channel rhodopsin (ChR2) mice, we optogenetically recruited cholinergic preganglionic synaptic properties in the interganglionic nerve and observed much larger amplitude EPSPs (20-40 mV) in the postganglionic cell. These EPSPs fatigued rapidly with inter-train periods of 5 sec, but not at 60 s intervals.

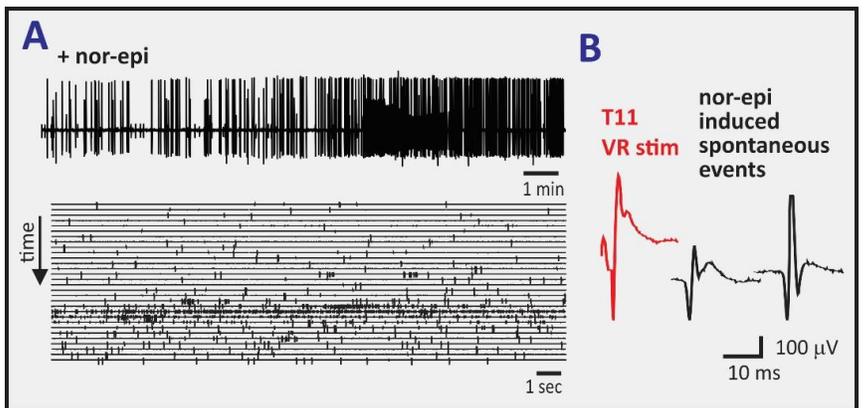
**Anatomical properties of tSPNs.** [Fig 4] Neurons within the sympathetic chain ganglia can be identified using TH and ChAT dependent reporter strains. Here, staining for GFP in a ChAT-GFP line shows the relative numbers of TH-immunolabeled adrenergic neurons to GFP-labeled cholinergic neurons (Fig 4A). Concomitant staining for CGRP positive primary afferents shows an interganglionic nerve composition with mutually exclusive adrenergic cholinergic CGRP+ axons (Fig 4B).

We have begun to take advantage of a TH-Cre lines with notable sparse labeling<sup>20</sup>. Strikingly, we saw that, while TH+ neurons in rostral thoracic ganglia contain dendrites, there is little evidence of dendrites in caudal thoracic chains (Fig 4C). The presence or absence of dendrites is an important factor in considerations of tSPN synaptic and firing properties in the modeling studies proposed. High magnification Confocal reconstructions suggest that adrenergic SPNs are synaptically interconnected (Fig 4D).

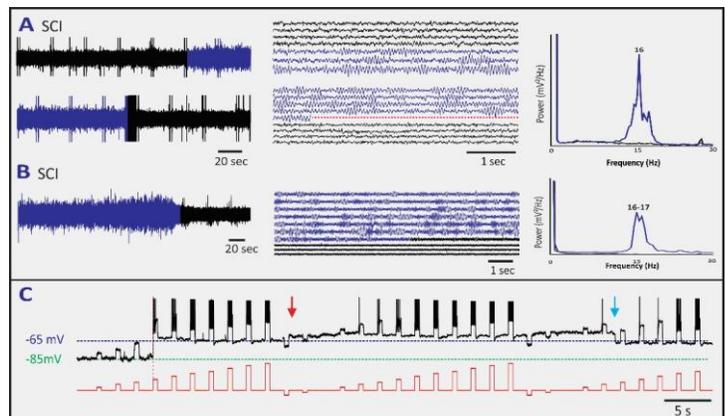


**Figure 5. Mean SPN cell diameter increases after SCI.** Distribution of TH+ neurons in T3 ganglia of sham (gray) and 3 wk spinalized mice (red).

**Plasticity after SCI. [Figures 5-8]** Cell diameters of TH<sup>+</sup> neurons in the T3 thoracic chain ganglia were clearly increased in the after SCI (Fig 5). In recordings from the T12 ganglia from other SCI mice, 3 of 4 animals expressed persistent oscillatory activity that was between ~10-20Hz and long-lasting (seconds to over 30 minutes; Fig 6AB). Strikingly, spontaneous bursting events acted as switches between oscillatory and quiescent states, consistent with an induction of membrane bistability. Only 1/27 control mice exhibited this behavior (and only in atropine). 10 Hz ventral root stimulation evoked long-lasting bouts of increased activity in 2/4 SCI mice but never in control mice (0/27) (Fig 7). Induction of activity increases were dependent on activation preganglionic nAChRs (mecamylamine sensitive). How bistability induced in individual tSPNs can synchronize to induced network oscillations is uncertain. However, synaptic interactions between tSPNs is one possibility given observation of putative somatic adrenergic synaptic interconnections (Fig 8D). The consequence is reinforcement of adrenergic excitability by ongoing activity. For example applied norepinephrine leads to the emergence of population synchronous spontaneous spikes within the T12 ganglion that were of comparable magnitude to evoked responses following high threshold electrical stimulation of the T11 ventral root (Fig 9). Additional extra-synaptic autocrine and paracrine release of transmitters, possibly a prevalent feature of sympathetic ganglia<sup>21</sup> may also contribute to population synchronization.



**Figure 8. Adrenergic recruitment of spontaneous activity in T12 chain ganglion. (A)** Top. Continuous recording following application of nor-epinephrine (100 μM). Bottom. Raster of same period showing 20 sec epochs of every 30 sec (period of evoked responses removed). **(B)** Ventral root stimulation-evoked response magnitude compared to emergent spontaneous events in the presence of nor-epinephrine.



**Figure 6. Long-lasting oscillation preferentially emerge after SCI.** Transitions between quiescent and oscillatory states were observed in 3/4 SCI and 1/27 control mice. **(A & B)** Show the emergence of oscillatory behavior in mice spinalized 5 or 6 days earlier. In **(A)** oscillations terminated following a spontaneous large burst of activity (bottom row). In **(B)** oscillations were induced following 10Hz stimulation. In both animals oscillations were at 16-17 Hz (right panels). **(C)** Membrane bistability as possible cellular equivalent to population oscillatory responses. T5 postganglionic neuron. Current ramps were delivered of different size and polarity as shown. A depolarizing pulse (at red vertical dotted line) led to a shift from a hyperpolarized (-85mV) to a more depolarized state (-65mV). Note also that the cell subsequently may have depolarized to a third excitable state on rebound from a hyperpolarizing pulse ~25s later before (red arrow), then spontaneously shifting back down to the first excitable state (cyan arrow). Spikes are shown truncated.



**Figure 7. Short train of preganglionic activity leads to prolonged postganglionic firing after SCI. (A & B)** Recording 5 or 6 days post-SCI, respectively. Dotted lines denoted 10Hz/5-pulse epochs of dorsal root (DR - red), ventral (VR - green) or co-stimulation (both - cyan). 10 Hz VR stimulation increased postganglionic firing that was abolished subsequent to application of the nAChR antagonist mecamylamine (50 μM; right panels). **(C)** Unlesioned mice never showed prolonged spiking after VR stim (0/27 mice). Trains were delivered every 30 sec.

#### 4) other achievements.

- Submitted a CRCNS grant application with a computational modeler as co-PI to try novel approaches that would hasten discovery while minimizing need for animals
  - Specific aims page is reproduced below

#### SPECIFIC AIMS

Sympathetic postganglionic neurons (SPNs) represent the final common sympathetic motor output. Thoracic SPNs (tSPNs) located in paravertebral chain ganglia receive convergent input from preganglionic neurons, providing the dominant sympathetic control of vascular function in the trunk and upper extremities. Given their strategic nodal site in autonomic signaling to body, any plasticity in tSPNs is likely to be of high significance. Yet tSPNs are inaccessible for *in vivo* study, so operational principles are inferred from studies in cervical<sup>1-3</sup> and lumbar chain ganglia<sup>4,5</sup>. To date, only 3 *in vitro* studies have revealed tSPN electrophysiological properties<sup>6-8</sup>, and there are still no accurate recordings of their cellular integrative properties or underlying recruitment principles.

We undertook THE FIRST PHYSIOLOGICAL STUDIES ON CAUDAL THORACIC CHAIN GANGLIA IN THE ADULT MOUSE by developing an *ex vivo* preparation with intact segmental preganglionic and rostrocaudal interganglionic connections<sup>9,10</sup>. We also obtained the FIRST WHOLE CELL RECORDINGS OF tSPN synaptic and cellular properties<sup>11</sup>. These data are a critical prerequisite to modeling studies, as observed synaptic integrative and firing properties are fundamentally different than previously observed with sharp electrodes due to impalement injury<sup>3,8</sup>. Our results already support tSPNs as overtly unique compared to SPNs characterized elsewhere. Here, we interleave experimental testing with modeling to understand tSPN recruitment principles and their integrative properties [SA1] as an essential launchpad to interrogate mechanisms that generate persistent bouts of hyperexcitability after spinal cord injury (SCI) [SA2].

**[SA1] Hypothesis.** tSPNs have heterogeneous synaptic, cellular, and network properties, and are active participants in input-output recruitment strategies. We tuned parameters obtained from bullfrog SPNs<sup>12,13</sup> with our whole-cell recordings to generate a conductance-based computational model having linear f-I curve and spike frequency adaptation. We will generate population models of input-output relations starting with thoracic T5 and T12 ganglia. Both receive predominant preganglionic input from their segmental ventral roots and their axon numbers can be obtained from respective white rami. Combined with counts of total tSPNs in these ganglia, we will pair experiments and modeling conditions to study population recruitment principles and integrative properties sculpting tSPN output. *Experiments* will involve testing orderly recruitment of preganglionic axons (in ChAT::CHR2 mice)<sup>14</sup> by varying blue light intensity while recording individual and population tSPN responses to understand recruitment order of individual tSPNs relative to recruited population responses. *Modeling* will explore the rules of population recruitment relative to; (a) the fraction of preganglionic axons recruited, and (b) indexed over a range of frequency-dependent synaptic fatigue responses. tSPN population output will be compared when cellular properties are modeled as uniform [homogeneous] vs. having an experimentally determined synaptic input range and cell excitability [heterogeneous].

**[SA2] Hypothesis.** tSPNs convert from linear to non-linear gain amplifiers after SCI. Somatic output-encoding motoneurons are bistable and can alter output gain via recruitment of dendritic plateau potentials<sup>15</sup>. Strikingly, we observed that most tSPNs lack dendrites and rarely exhibit bistability. However, population oscillatory activity was triggerable after SCI<sup>9</sup> supporting emergent membrane bistability coincident with dendritic sprouting<sup>16</sup>. ① **Experiments.** Whole-cell recordings will compare differences in the response properties of tSPNs with (T2-3) and without dendrites (T5&12). Voltage and current clamp step and ramp protocols will test for activation of persistent inward currents (PICs) and expression of membrane bistability. Synaptically-evoked recruitment of tSPNs will determine whether bistability is synaptically-triggered while dye labeling will relate morphology to observed cellular responses. We will similarly compare caudal tSPN responses in control mice and after high thoracic SCI to assess whether bistability corresponds to dendritic sprouting, and also leads to persistent population voltage oscillations. ② **Modeling** with a two compartment neuron model that generates membrane bistability via insertion of a dendritic PIC will test the consequence of triggerable bistability to overall population output using an otherwise identical model to that in SA1. ③ **Modeling** will generate hypotheses on factors contributing to autonomic dysreflexia (AD) - a sudden, persistent sympathetic hyperactivity seen in people (and rodents) after high thoracic SCI - by testing whether increases in synaptic strength (sprouting) and/or dendritic PICs (dendritic growth) alters tSPN cellular/population recruitment patterns consistent with AD. The model will also implement strategic parameter changes that simulate drug actions at known binding sites to test whether tSPNs are a plausible locus of AD therapeutics. ④ **Experiments** will then use the *ex vivo* model to directly test for concordant drug-induced changes in tSPN excitability.

**Overall significance.** If successful, combined approaches will have finally uncovered the operational principles governing the final neural command pathways regulating vascular tone by generating an accurate and detailed electrophysiological database of thoracic chain ganglia. Aberrant increases in tSPN gain could lead to sympathetic hyperactivity in various autonomic disorders. SPN dysfunction may be pivotal to impaired function in cardiac arrhythmias, diabetes, oxidative stress, and essential hypertension<sup>1,3,17-19</sup>. A

database amenable to realistic modeling studies should be transformative to the field. Insights derived from probing the network parameter space for putative neural bases of emergent dysfunction, could catalyze novelty in both experimental testing and in drug discovery-based therapeutic considerations.

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*Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

- c. What opportunities for training and professional development has the project provided?
  - o *Two individuals were sent to Chicago this October to present their data as a poster in at a satellite symposium in conjunction with the Annual Society for Neuroscience Meeting.*
- d. Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.
  - o *Continue investigations as proposed with more focus on anatomical and cellular plasticity*

#### 4. IMPACT:

*Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- o **What was the impact on the development of the principal discipline(s) of the project?**
  - *Nothing to Report*
- o **What was the impact on other disciplines?**
  - *Led to a CRCNS application with a computational neuroscientist*
- o **What was the impact on technology transfer?**
  - *Nothing to Report*
- o **What was the impact on society beyond science and technology?**
  - *Nothing to Report.*

#### 5. CHANGES/PROBLEMS:

*Nothing to Report*

#### 6. PRODUCTS:

*Nothing to Report*

Publications, conference papers, and presentations

**Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.*

1. M Halder and S Hochman. Early insight into emergence of spontaneous synchronous oscillatory activity in isolated thoracic chain ganglia after SCI. Pre-meeting on rhythmic motor circuits. 10/16/2015.
2. M McKinnon, M Choi, S Hochman. Characterization of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia. Pre-meeting on rhythmic motor circuits. 10/16/2015.

#### 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

- *Hannah Choi – 5% effort – Honor’s research Project*
- *Mallika Halder – 75% effort – research specialist*
- *Michal McKinnon – 90% effort – graduate student*
- *Michael Sawchuk, - 75% effort - lab manager*

e. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- *Nothing to Report*

f. What other organizations were involved as partners?

- *Nothing to Report*

## 8. SPECIAL REPORTING REQUIREMENTS

## 9. APPENDICES:

g. abstracts,

1. **M Halder and S Hochman.** Early insight into emergence of spontaneous synchronous oscillatory activity in isolated thoracic chain ganglia after SCI. *Pre-meeting on rhythmic motor circuits.* 10/16/2015.

Department of Physiology, Emory University School of Medicine, Atlanta, GA

Paravertebral sympathetic postganglionic neurons (SPNs) located in thoracic chain sympathetic ganglia represent the predominant sympathetic control of vascular function in the upper and middle extremities. Loss of descending control of autonomic function as occurs after spinal cord injury (SCI) leads to a variety of autonomic changes that could arise in part from changes within paravertebral sympathetic chain SPNs. We developed an in vitro adult mouse model to examine evoked population responses in these ganglia following stimulation of segmental sympathetic preganglionic and primary afferents axons before and after spinal cord injury. The approach retains the adult mouse thoracic sympathetic chain ganglia in situ with connections to dorsal root ganglia, dorsal roots and ventral roots. Dorsal roots were stimulated to examine primary afferent evoked responses, and ventral root stimulation was used to recruit axons of sympathetic preganglionic neurons. Recordings of population responses in individual ganglia with suction electrodes attached to the inter-ganglion root were undertaken to characterize the plasticity in sympathetic chain ganglia after spinal cord injury. While preliminary and very early after SCI, sympathetic chain ganglia from both these animals underwent spontaneous bursts of activity that initiated long- lasting membrane oscillations. When recording from the T12 ganglia, in animals that had undergone T2 spinalization previously, we observed that spontaneous activity could initiate or abolish ongoing persistent oscillatory activity that was long-lasting (seconds to over 30 minutes). Ventral root stimulation could also abolish ongoing oscillatory behavior. Oscillation frequencies were between ~10-20Hz. Strikingly, bursts acted as switches between oscillatory and quiescent states, suggestive of a SPN circuit-based capacity for the induction of membrane bistability. In comparison, only 1 of 25 control animals exhibited this behavior, and only in the presence of atropine. Furthermore, in the animal that had undergone SCI 5 days prior, ventral root stimulation evoked long- lasting bouts of spontaneous activity that was abolished following nicotinic receptor block with mecamylamine, supporting activity induced by preganglionic nicotinic actions on postganglionic neurons. Studying these neuroplastic changes in sympathetic postganglionic neurons following SCI will allow us to understand how synaptic alterations contribute to autonomic dysfunction. Further studies are proposed. Supported by the Department of Defense (Awards #25530)

2. **M McKinnon, M Choi, S Hochman.** Characterization of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia. *Pre-meeting on rhythmic motor circuits.* 10/16/2015.

Thoracic intraspinal **preganglionic** neurons constitute the entire CNS sympathetic output. They project to sympathetic **postganglionic** neurons within autonomic ganglia. A major function of sympathetic paravertebral chain ganglia neurons is to maintain vasomotor tone. While the functional properties of cervical and lumbosacral paravertebral ganglia neurons have been characterized, little is known about the functional properties of neurons within thoracic paravertebral ganglia. We developed an approach that allows for whole-cell patch clamp recordings in intact thoracic ganglia to characterize cellular and synaptic properties.

We recorded from 5 cells, 2 of which were of adequate quality to characterize passive membrane properties. Preliminary findings from whole-cell recordings (n=2) in fifth right thoracic ganglia show that cells have input resistance around 485M $\Omega$ , resting membrane potential -55 to -50 mV, and membrane time constant of 85 ms. Threshold voltage was typically 10 mV higher than resting membrane potential, action potentials displayed after-hyperpolarization and some cells displayed post-inhibitory rebound. Maximal firing rates observed in response to depolarizing current steps ranged from 14-17 spikes/sec. During intracellular depolarization, firing rate increases with increased current injection and cells sustain tonic firing.

Preliminary examination of nicotinic acetylcholine receptor (nAChR) activation was studied in one neuron from the fifth right thoracic ganglion. When perfused with 100uM acetylcholine, this cell

depolarized from -60mV to -40mV and was observed to fire at a maximal instantaneous firing rate of 15 Hz. Spike frequency adaptation was also observed; spiking gradually stopped as membrane potential repolarized to -30mV around 40s after initiation, presumably by receptor desensitization. Choline also led to a modest membrane depolarization. In both cases, depolarizing response was attenuated in the presence of nAChR antagonist, hexamethonium.

Patch clamp recordings in combination with optogenetics in another cell from the seventh right thoracic ganglion from a ChAT-ChR2 mouse suggested that postganglionic neurons receive massive cholinergic input from preganglionic neurons. Repetitive (10 Hz) optical stimulation of cholinergic preganglionic axons elicited a large EPSP in the postganglionic neuron (20 mV). The EPSP was usually suprathreshold and accompanied by an action potential. The response fatigued dramatically to repetitive stimuli. This seems to indicate that presynaptic release of acetylcholine attenuates rapidly on the order of 100-300 milliseconds. Together, these data suggest that presynaptic release of acetylcholine by preganglionic neurons is the rate-limiting step for transmission in thoracic paravertebral ganglia.

By using a ChAT-cre x LacZ reporter mouse which labels a sparse subpopulation of cholinergic preganglionics, we were able to observe individual synaptic arborizations. Each arborization within thoracic paravertebral ganglia was observed to surround a single postganglionic neuron with several (10-20) large axosomatic synapses. This organizational principle, a one-to-one connection between pre- and postganglionic neurons, is in line with observations from other sympathetic ganglia.



EMORY UNIVERSITY

# EARLY INSIGHT INTO THE EMERGENCE OF SPONTANEOUS SYNCHRONOUS OSCILLATORY ACTIVITY IN ISOLATED THORACIC CHAIN GANGLIA AFTER SCI

Mallika Halder, Michael McKinnon, Michael Sawchuk, Shawn Hochman  
Physiology Department at Emory University School of Medicine, Atlanta, Georgia USA.



EMORY UNIVERSITY SCHOOL OF MEDICINE

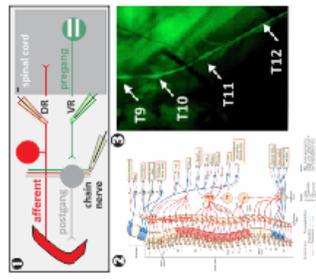
## INTRODUCTION

Postnatal sympathetic ganglia control autonomic function in the upper and middle extremities. Loss of descending control of their sympathetic outflow leads to a hyperactive sympathetic outflow. The emergence of spontaneous oscillations, particularly lower and thoracic MyoTonicus, including autonomic dysreflexia, occurs in individuals with lesions above T4 that compromise descending control over the lower and mid thoracic sympathetic outflow. It is not clear how the development of the mid thoracic sympathetic outflow is affected in SCI.

We speculate that observed oscillations occur in part as a change within the sympathetic outflow, specifically in the thoracic sympathetic outflow. More caudal sympathetic outflow is particularly susceptible to the autonomic dysreflexia. We developed an *in vitro* model to study thoracic sympathetic chain ganglia in vitro. It has attached ventral roots for afferent and efferent sympathetic outflow, as well as intact dorsal root ganglia and distal nerves. We used intracellular extracellular recordings to provide the first insight into ganglia in SCI.

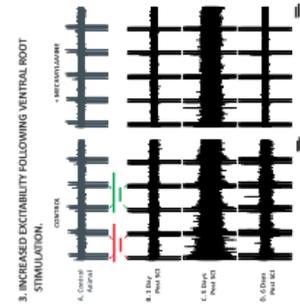
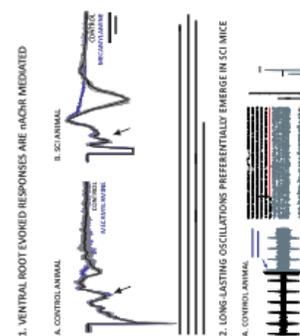
## in vitro Mouse Model

- The thoracic sympathetic outflow of C57BL/6J mice (P10) was isolated and placed in a recording chamber perfused with artificial cerebrospinal fluid.
- The ganglia were exposed by the dorsal laminectomy and the thoracic outflow was isolated to avoid dorsal root ganglia.
- Stimulating suction glass electrodes were placed on ventral roots to stimulate sympathetic outflow.
- Recordings were made from thoracic sympathetic outflow (T9-T12) to assess sympathetic outflow between 1 control and 1 SCI animal at T2, T3, T4, T5, T6, and T7 post SCI.

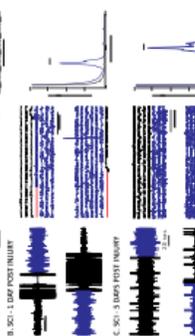


## RESULTS

### FEATURES OBSERVED AFTER SCI



### CHAIN ANATOMY



### PRELIMINARY CHARACTERIZATIONS SUGGESTS POSSIBLE CHANGE IN CELL DIAMETERS & POPULATION TYPES AFTER SCI



## DISCUSSION

We developed an *in vitro* mouse model that allows for the study of thoracic sympathetic chain ganglia in SCI. The specific, preganglionic and CGRP+ efferent axons were observed in thoracic sympathetic chain ganglia. Preliminary differences in cell sizes and populations in thoracic sympathetic chain ganglia before and after SCI suggest that SCI affects the thoracic sympathetic chain ganglia. Cell diameters of ganglia are smaller than thoracic ganglia. Vertical root evoked responses in T12 sympathetic chain ganglia are partially mediated and can be blocked with NMDA antagonists.

Long lasting spontaneous oscillations induced by spontaneous events, mechanical and high intensity 10 Hz electrical stimulation were observed in thoracic sympathetic chain ganglia compared to the T12 control animals. Frequency of oscillatory behavior ranged from 10-12 Hz. Oscillations were blocked on or off with spontaneous events.

Adjusting long duration oscillations induced by 10Hz electrical stimulation, we observed an increase in oscillation frequency in an animal 6 days after SCI.

The ventral root that had dorsal root stimulation increases excitability via NMDA mechanisms 5 and 6 days after injury. Increased spiking can be blocked with NMDA antagonists. This suggests that oscillations showed an increase in excitability following SCI.

Intracellular recordings from a T5 ganglia cell show evidence of a stable state change. The possibility that a group of ganglia in a SCI animal is highly active and with the membrane property of instability and offers an explanation for thoracic sympathetic chain ganglia in spontaneous oscillations. This is the way one might think about autonomic dysreflexia. The model has no spinal cord or brain stem but can still show high frequency spikes.

## REFERENCES

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- Ulfhake, J. W., Burns, C. D., & Tye, S. W. (2004). Involvement of sympathetic neurons in the guinea pig thoracic chain. *J Physiol*, 556, 187-196.
- Jahng, P. R., & Gohles, L. L. (2004). Electrophysiological and morphological density of mouse sympathetic neurons. *Journal of Neurophysiology*, 92, 2170-2176.

## ACKNOWLEDGEMENTS

Supported by Department of Defense SCI-30225

## SUMMARY OF SPINALIZED ANIMALS TESTED IN THESE STUDIES

Animal ID	Sex	Age	SCI Level	Post-SCI Days	Spontaneous Oscillations	Long-Lasting Oscillations
1	Male	10	T12	1	Yes	No
2	Female	10	T12	1	Yes	No
3	Male	10	T12	1	Yes	No
4	Female	10	T12	1	Yes	No
5	Male	10	T12	1	Yes	No
6	Female	10	T12	1	Yes	No
7	Male	10	T12	1	Yes	No
8	Female	10	T12	1	Yes	No
9	Male	10	T12	1	Yes	No
10	Female	10	T12	1	Yes	No
11	Male	10	T12	1	Yes	No
12	Female	10	T12	1	Yes	No
13	Male	10	T12	1	Yes	No
14	Female	10	T12	1	Yes	No
15	Male	10	T12	1	Yes	No
16	Female	10	T12	1	Yes	No
17	Male	10	T12	1	Yes	No
18	Female	10	T12	1	Yes	No
19	Male	10	T12	1	Yes	No
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21	Male	10	T12	1	Yes	No
22	Female	10	T12	1	Yes	No
23	Male	10	T12	1	Yes	No
24	Female	10	T12	1	Yes	No
25	Male	10	T12	1	Yes	No
26	Female	10	T12	1	Yes	No
27	Male	10	T12	1	Yes	No
28	Female	10	T12	1	Yes	No
29	Male	10	T12	1	Yes	No
30	Female	10	T12	1	Yes	No
31	Male	10	T12	1	Yes	No
32	Female	10	T12	1	Yes	No
33	Male	10	T12	1	Yes	No
34	Female	10	T12	1	Yes	No
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36	Female	10	T12	1	Yes	No
37	Male	10	T12	1	Yes	No
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41	Male	10	T12	1	Yes	No
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