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ABSTRACT 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?

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15. SUBJECT TERMS

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Contents

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

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.*

Characterizing thoracic chain sympathetic postganglionics		
Major Task 1a: Convergence and divergence	**months**	**% completion/ Completion dates**
Subtask 1: Segment specific properties	1-6	75%
Subtask 2: Pharmacology	7-12	*75%*
Subtask 3: Breeding/crossing transgenic mice and spinalizations	1-36	6months behind target
Subtask 3: Establish intracellular recording techniques	3-18	100%
Major Task 1b: Convergence and divergence	**months**	
Subtask 1: Incorporation of optogenetic approaches for selective activation of neuron populations	12-18	100%
Milestone(s) Achieved: Understanding of synaptic organization in uninjured mice and ability to use optogenetics to selectively activate afferent and efferent fiber populations		
Intracellular recordings and optogenetics		
Major Task 2: Characterize mechanisms responsible for dysautonomia after spinal cord injury using intracellular recordings and optogenetics	**months**	**% completion/ Completion dates**
Subtask 1: Physiological plasticity in preganglionic-postganglionic interactions assessed using optogenetics	18-36	5%
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	10%
Milestone(s) Achieved: Demonstration of important contribution of thoracic sympathetic chain to SCI-induced autonomic plasticity and forward insight into therapeutic interventions for future study		
Data analysis and publications		
Major Task 3: Data analysis and publications	**months**	**% completion/ Completion dates**
Subtask 1: Data analysis	6-36	35%
Subtask 2: Manuscript writing and submission	24-36	10%
Milestone(s) Achieved: Dissemination of scientific results.		

b. What was accomplished under these goals?

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

Accomplishments under specific sections are described below followed by an overall annual summary that synthesizes these accomplishments. Please refer to figures in the overall summary as needed.

1a.1: Segment specific properties
Methods/experiment: Mice are euthanized (.2mL 50% urethane) and thoracolumbar spinal column quickly removed. The vertebral column is cut longitudinally, both dorsally and ventrally, and spinal roots are severed to remove spinal cord. Remaining vertebral column and ribs are trimmed to include only the thoracic region*. The tissue is pinned down in a sylgard recording chamber and suction electrodes are positioned to stimulate various thoracic ventral roots and record from various thoracic ganglia.
Progress/results: Extracellular recordings show that there is a convergence onto individual ganglia. For example, stimulating T4-T11 ventral roots results in activity in the T11 ganglion (Fig. 7A in *overall summary* below). If we repeat this with many ganglia, a more complete picture emerges, showing that there is also significant divergence of ventral root input to thoracic ganglia (Fig. 7C in *overall summary* below). We aim to repeat these trials using optical stimulation of ventral roots. We are currently training an undergraduate student to help with these experiments.

1a.2: Pharmacology
Methods/experiment: Dissected vertebral column described in the methods section above is pinned down in recording chamber with stimulating suction electrodes on various ventral roots and a recording electrode on thoracic ganglia. We have been testing for synaptic transmitter identity by applying glutamatergic, cholinergic, nitrergic, purinergic and adrenergic ionotropic receptor antagonists to the recording chamber.
Progress/results:
Extracellular Recordings. We have found evidence for a contribution from glutamatergic, nitrergic and cholinergic transmission in both ventral root and dorsal root evoked responses. Postganglionic transmission is thought to occur via nicotinic acetylcholine receptor subunits. We have conducted experiments with nAChR antagonists that act on different receptor subunits and have found reduction from baseline synaptic transmission. Our next step is to increase the sample size.
Intracellular Recordings. Experiments are underway to analyze the effects of various channel blockers on intrinsic membrane currents in intracellular recordings from individual neurons.

1a.3: Breeding/crossing transgenic mice and spinalizations
Methods/experiment: Standard animal husbandry
Progress/results: We currently have a healthy colony of ChAT-IRES-Cre::ChR2 mice available for performing in vitro optogenetic studies. We believe these mice will be more suitable than the BAC transgenics we previously used due to the more precise nature of their transgene insertion. These mice are used for all studies, with the exception of subtask 2.2. Subtask 2.2 will require the generation of Advillin::ChR2 mice to study afferent-postganglionic interactions. We are in possession of the requisite mouse strains, but have refrained from crossing them until other subtasks have neared completion.
Spinalizations are behind schedule. The difficulty of caring for injured mice compounded with the relatively low success rate of our intracellular recording technique has slowed progress in this area. However, we plan to begin spinalization surgeries in earnest in early 2017. We hired and trained a new technician to help with animal care and have optimized our recording technique to moderately improve success rate.

1a.4: Establish intracellular recording techniques
Methods/experiment: Starting with the preparation to isolate the thoracic chain and after ribs and vertebrae are trimmed (see 1a.1 methods, *) the entire tissue is incubated at 37°C in collagenase for 1.5 hours. The tissue is then washed in physiological saline. Sympathetic chain is removed by severing rami and transferred to a recording chamber. Chain is pinned down in Sylgard, connective tissue is removed by scraping lightly with an insect pin, and recorded using standard patch clamp technique.
Progress/results: We are currently able to achieve acceptable recordings from most mice used in experiments, with recordings lasting at least 5-10 minutes. This is sufficient time to characterize basic cellular properties (i.e. input resistance, cell capacitance, basic firing properties, etc.) However, longer recordings are required to characterize convergent synaptic input properties and to study membrane current pharmacology. To date, high fidelity, long lasting recordings are admittedly rarer. Much of this is due to connective tissue and glia that cover

neuronal surfaces and make stable seals difficult to achieve. To compensate, we have simply increased the number of mice from which we attempt to record. Progress overall has been steady, but still slower than we had hoped.

1b.1: Incorporation of optogenetic approaches for selective activation of neuron populations
Methods/experiment: We have developed a laser-diode based stimulator which allows for optical activation of preganglionic axons in ChAT::ChR2 mice. Light can be directed to illuminate ventral roots (primarily for extracellular recordings), interganglionic nerve, or thoracic ganglia.
Progress/results: Evoked synaptic response fatigues due to repeated stimulation, and takes seconds to recover (Fig. 6C in *overall summary* below). When the entire ganglion is illuminated, eliminating any significant propagation delay, an interesting relationship emerges between excitatory postsynaptic current (EPSC) amplitude and latency (time between optical stimulus and the start of an evoked EPSC). Shorter latency corresponds to greater amplitude (Fig. 6D in *overall summary* below). When the interganglionic is illuminated, we have shown that different preganglionics are activated at different thresholds (Fig. 6E in *overall summary* below). We have also shown that a single cell receives input from multiple spinal cord segments (Fig. 7D in *overall summary* below).

2.1: Physiological plasticity in preganglionic-postganglionic interactions assessed using optogenetics
Methods/experiment: Methods described in 1b.1 are repeated in spinal cord injured mice.
Progress/results: Progress has been slow in this area. Tissue from injured mice appears to be more difficult to patch, i.e. high resistance seals are hard to achieve and recordings are "leaky." In light of this observation, we intend to stain the tissue for extracellular matrix components (collagen, chondroitin sulfate proteoglycans) to test the hypothesis that the extracellular matrix becomes denser after SCI. As stated previously, we have hired a new technician to help streamline the injury and recording process.

2.2: Physiological plasticity in afferent-postganglionic interactions assessed using optogenetics
Methods/experiment: N/A
Progress/results: N/A

2.3: Physiological plasticity in preganglionic-afferent interactions assessed using optogenetics
Methods/experiment: N/A
Progress/results: N/A

2.4: Intracellular recordings of synaptic and cellular plasticity in membrane properties; demonstration of membrane bistability
Methods/experiment:
Progress/results: SCI may induce greater frequency of spontaneous synaptic events. However, we currently have n=2 so this must be replicated before we can say this with confidence.

3.1: Data analysis
Methods/experiment: Data is analyzed in Clampfit, MATLAB, and/or Excel.
Progress/results: Basic cellular properties (input resistance, membrane capacitance, time constant, firing rate) have been analyzed. Analysis of synaptic properties are in progress.

3.2: Manuscript writing and submission
Methods/experiment: N/A
Progress/results: Manuscript writing is in progress. The abstract and methods sections are essentially complete. The results section is still in progress.

Overall summary: Sympathetic postganglionic neurons (**SPNs**) represent the final common sympathetic motor output. Thoracic SPNs (**tSPNs**) located in paravertebral chain ganglia control vasomotor function in trunk and upper extremities (*Fig 1A*). Their plasticity is likely to be of high clinical significance. Yet tSPNs are inaccessible for *in vivo* study, so operational principles are inferred from studies in cervical and lumbar chain ganglia (Percy and Krier, 1987; Bratton et al., 2010; Campanucci et al., 2010; Rimmer and Horn, 2010; Springer et al., 2015). To date, there are only 3 *in vitro* studies on tSPN physiological properties (Blackman and Purves, 1969; Lichtman et al., 1980; Jobling and Gibbins, 1999), and no accurate recordings of their cellular

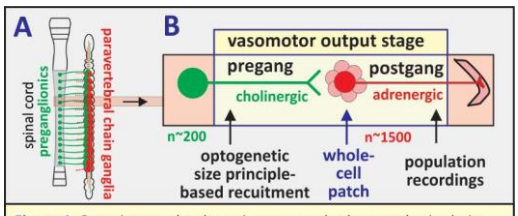


Figure 1. Overview on the thoracic paravertebral sympathetic chain ganglia and experimental design. **(A)** Chain ganglia lie ventrolateral to the spinal column and are interconnected by an interganglionic nerve (not shown). **(B)** Example single segment connectivity and experimental design *(pre- and postganglionic neuron #s are speculative*).

integrative properties or underlying recruitment principles. We developing an *ex vivo* adult mouse preparation with intact segmental preganglionic and rostrocaudal interganglionic connections and obtained the FIRST WHOLE CELL RECORDINGS OF tSPN synaptic and cellular properties. Observed synaptic integrative and firing properties were fundamentally different than previously observed with sharp electrodes due to impalement injury (Jobling and Gibbins, 1999; Springer et al., 2015), and finally provide the critical prerequisite to understand tSPN cellular integrative and recruitment principles as a launchpad to interrogate mechanisms that generate abnormal increases in excitability including after spinal cord injury (**SCI**).

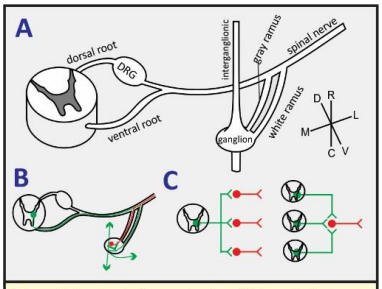


Figure 2. Anatomical organization of thoracic chain pre- and postganglionic elements. **(A)** Ganglia are connected to the spinal nerve via white and gray rami communicans, and to eachother through the interganglionic nerve. **(B)** Preganglionic axons exit spinal cord via the ventral root and enter the sympathetic chain via the white ramus communicans. Axons may synapse onto tSPNs directly, and travel up or down the chain any number of segments, or directly exit the chain to innervate prevertebral ganglia (mesenteric, celiac). **(C)** A single preganglionic neuron may diverge to innervate several SPNs. Likewise, several preganglionic neurons from different spinal segments may converge to innervate the same SPN.

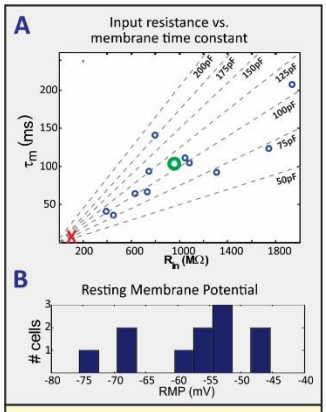


Figure 3. Whole-cell recordings of tSPN passive membrane properties. **(A)** Comparison of input resistance (R_{in}), membrane time constant (τ_m), and membrane capacitance (n=11). Resistance and tau were calculated by fitting an exponential to hyperpolarization transients. Capacitance was calculated from τ_m=RC. Mean value represented by **green O**. Mean value reported by Jobling & Gibbins (1999) is represented by **red X**. **(B)** Resting membrane potential (RMP) for SPNs (n=11). [uncorrected for liquid junction potentials].

Most thoracic paravertebral sympathetic postganglionic neurons (**tSPNs**) control vasomotor function in upper and middle extremities of the trunk. This includes vascular supply to integumentary, cardiorespiratory and digestive systems. Whereas prevertebral sympathetic ganglia are typically associated with one or more visceral organs in a discrete location, chain ganglia can be thought of as a distribution system for sympathetic activity that must span the body vasculature.

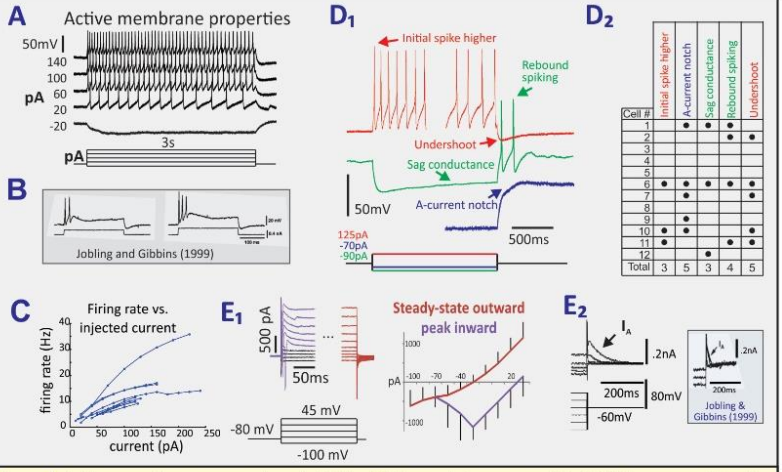


Figure 4. Active membrane properties of tSPNs. **(A)** Current steps reveal that tSPNs are capable of sustained firing. **(B)** As shownin panel from Jobling and Gibbins (1999), sharp electrode recordings only ever observed phasic firing. **(C)** Spike frequency vs. injected current relations (*f*-I curves) (n=10). Profile is remarkably similar for most cells. **(D_1)** Current clamp. (Left) Representative traces showing evidence of several votage-gated conductances in some cells. Red trace: Initial spike is ~5-10mV higher than spikes that follow, and voltage dips below baseline temporarily after release from depolarization. Green trace: Rebound spiking is observed after release from moderate hyperpolarization. Green trace: Sag conductance putatively due to activation of Ih channels. Blue trace: An A-current notch. Each trace comes from a different cell. **(D_2)** Table showing relative abundance of aforementioned voltage features. **(E_1)** Voltage clamp. (Left) Representative traces showing peak inward and steady state outwards currents generated at various voltage steps. (Right) Population I-V plot of steady state outward and peak inward currents (n=4). **(E_2)** Activation of I_A following membrane hyperpolarization is comparable to that reported previously (shown in grey box)

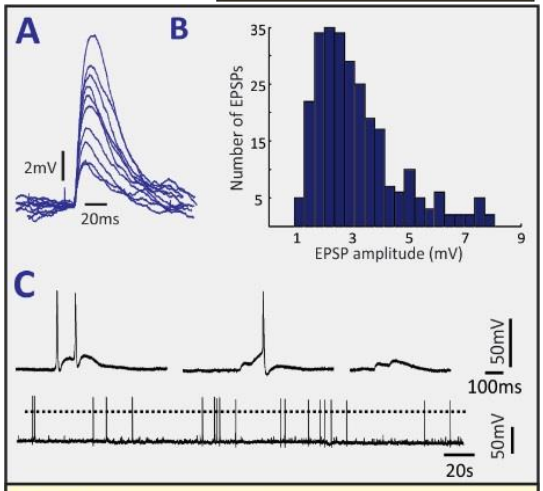


Figure 5. Spontaneous synaptic properties tSPNs include synaptic integration-based recruitment of spiking. **(A)** Overlaid traces of captured EPSPs in a spontaneously active cell. **(B)** Histrogram of events showing that EPSP amplitudes occupy a continuous range from 1 to 8mV. **(C)** Bottom - Representative trace from another spontaneously active cell. Top - Callouts showing EPSPs with temporal overlap, two of which led to spiking.

One important issue is whether sympathetic postganglionic neurons (**SPNs**) are driven directly from preganglionic neurons giving a simple chain of command. Alternatively, postganglionic neurons can act as integrators, making the information processing system more complex during recruitment. A summary of their anatomical organization is shown (***Fig 2***). They are presumably excited by sympathetic preganglionic neurons via the **'n+1' rule**. The 'n+1' rule states that the postganglionic neurons receive one (**1**) primary synapse from a preganglionic neuron, and several (**'n'**) secondary synapses which are smaller and have little effect on postganglionic firing properties. This organizational principle of recruitment is believed to dictate recruitment of postganglionics in superior cervical (Rimmer and Horn, 2010) and lumbar sympathetic chain ganglia (McLachlan, 2003). Consequently, SPNs have traditionally been considered to behave as 1:1 relays of preganglionic commands.

UPDATE. (A) Characterization of cellular properties in adult mouse thoracic paravertebral ganglia. [Fig 3 & 4] 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 12 cells deemed of good quality and obtained the following mean values ± SD: resting membrane potential (-57 ± 9 mV) [Fig. 4A], membrane resistance (985 ± 501 MΩ), and τ_m (99 ± 49 ms) [Fig. 4B]. 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 (Springer et al., 2015). Strikingly, our recorded properties differ substantially from sharp electrode recordings obtained from adult mouse tSPNs (Jobling and Gibbins, 1999). Our recorded membrane resistance is 4.5 fold higher and τ_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.

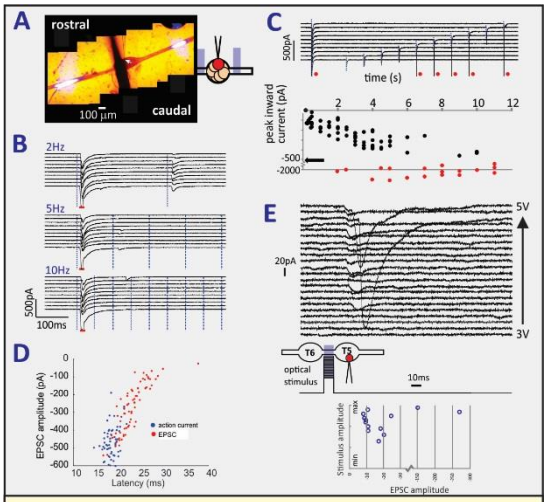


Figure 6. Evoked synaptic properties in tSPNs. **(A)** We selectively stimulated preganglionic axons using optogenetics to investigate cholinergicsynaptic properties (ChAT-IRES-cre crossed with cre-dependent ChR2-YFP mice). Preganglionic axons can be selectively stimulated from ventral roots (Fig 7D) or here in the thoracic interganglionic nerve. **(B)** Activation of preganglionic axons leads to a massive (~500pA) EPSC in the postganglionic cell. This response fatigues rapidly with repetitive 10Hz stimulation. **(C)** Rate of recovery after fatigue was characterized using a paired pulse protocol. Synaptic fatigue lasted several seconds before synapses fully recovered. **Bottom trace.** Relates interpulse interval with EPSC amplitude. Note various occasions where optogenetic recruitment of EPSPs led to action currents (these are denoted by red circles in both panels). **(D)** There is a robust relationship between event onset after optical pulse and the amplitude of the resultant EPSC in one cell. Shorter latency corresponds with higher amplitude and vice versa. Blue dots represent EPSCs that led to action currents, but for which the amplitude of the underlying EPSC could be easily measured; red dots represent EPSCs that did NOT elicit an action current.**(E)** interganglionic nerve was illuminated with varying optical intensity. Lower amplitude EPSCs appear to have a lower threshold of activation compared to larger EPSCs recruited with higher intensity.

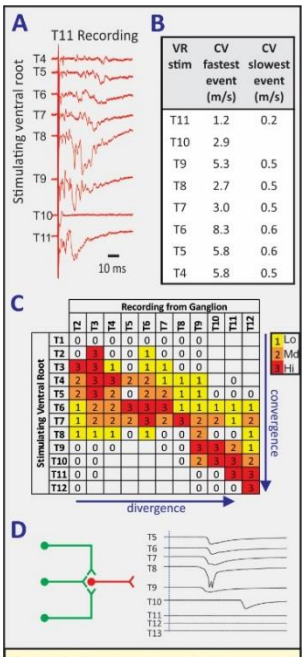


Figure 7. Convergence and divergence of inputs onto thoracic chain ganglia. **(A)** Extracellular recording of convergent input onto the T11 ganglion. **(C)** Conduction velocities range between 0.2-8.3 m/s - comparable to that observed in guinea pig (Blackman & Purves, 1969). **(C)** Map of convergence and divergence in another animal. Estimates of preganglionic convergence onto individual ganglia are shown as columns progressing from T2-T12 color-coded to projection magnitude. Preganglionic divergence is shown in rows. Patterns are comparable to guinea pig (Lichtman et al 1980). **(D)** Characterization of preganglionic convergence in an individual tSPN. Ventral roots from each segment indicated were stimulated, EPSCs were recorded from a T8 tSPN.

(B) <u>Synaptic and anatomical properties of thoracic sympathetic chain ganglia.</u>

<u>Synaptic properties of paravertebral neurons.</u> Spontaneous EPSP amplitudes occupy a continuous range. For the neuron shown, this was from 1-8mV (***Fig 5A,B***). Previous studies implied that due to the short time constant of postganglionic neurons, synaptic integration has a negligible effect on cell firing. Using patch clamp recordings, the measured time constant of cells is longer, so we observe that spontaneous coincident EPSPs often sum and lead to an action potential (***Fig 5C***).

Using (**ChAT**)::channel rhodopsin (**ChR2**) mice, we optogenetically recruited either cholinergic preganglionic axons in the interganglionic nerve or preganglionic synapses within thoracic ganglia (***Fig 6***). Activation of preganglionics by blue light illumination leads to a massive EPSC (~500pA) that fatigues rapidly with repetitive 10Hz stimulation (***Fig 6B***). As stimulation frequency increases, synaptic currents show considerable fatigue. This may indicate that presynaptic release of acetylcholine attenuates rapidly (on the order of 100-300ms) and this is the rate-limiting step for transmission in tSPNs. Rate of recovery after fatigue was also characterized using a paired pulse protocol (***Fig 6C***). The effects of synaptic fatigue are apparent even after 5 seconds of rest. We also obtained preliminary evidence for the existence of a size principle of recruitment. We observed a robust relationship between event onset after optical pulse and the amplitude of the resultant EPSC in one cell (***Fig 6D***). The aforementioned studies do not assess the validity of the 'n+1'-rule; results obtained with spontaneous events with Poisson distribution argue against the 'n+1'-rule (***Fig 5***), while high intensity optical axon recruitment elicited large and highly fatiguing synaptic actions that support the 'n+1'-rule. In order to resolve this issue, we measured graded intensity of optically stimulation and recorded EPSCs under voltage clamp (***Fig 6E***). Lower amplitude EPSCs appeared to have lower threshold of activation compared to larger EPSCs recruited with higher intensity illumination, but t effect was not linear.

Properties of preganglionic divergence and convergence onto thoracic chain SPNs was previously examined in guinea pig (Blackman and Purves, 1969; Lichtman et al., 1980). Spinal cord preganglionic neurons project axon collaterals intersegmentally for divergent actions on many tSPNs. Conversely, tSPNs within an individual ganglion

receive preganglionic convergent input from multiple spinal segments (Blackman and Purves, 1969; Lichtman et al., 1980). In the superior cervical ganglion, individual SPNs receive input on average from ~9 preganglionic neurons (Purves and Lichtman, 1985). In comparison, the firing of postganglionics in rodent lumbar ganglia may be 'normally' driven by 2 to 3 preganglionic neurons (Bratton et al., 2010), We began to undertake similar studies in the adult mouse and observed comparable patterns of convergence and divergence. Recruited of presynaptic events covered a comparable range of conduction velocities (Blackman and Purves, 1969)(***Fig 7A-C***). We have also begun to assess convergence properties onto individual tSPNs (***Fig 7D***).

Blackman JG, Purves RD (1969) Intracellular recordings from ganglia of the thoracic sympathetic chain of the guinea-pig. J Physiol 203:173-198.

Bratton B, Davies P, Janig W, McAllen R (2010) Ganglionic transmission in a vasomotor pathway studied in vivo. J Physiol 588:1647-1659. doi: 1610.1113/jphysiol.2009.185025. Epub 182010 Mar 185022.

Campanucci V, Krishnaswamy A, Cooper E (2010) Diabetes depresses synaptic transmission in sympathetic ganglia by inactivating nAChRs through a conserved intracellular cysteine residue. Neuron 66:827-834. doi: 810.1016/j.neuron.2010.1006.1010.

Jobling P, Gibbins IL (1999) Electrophysiological and morphological diversity of mouse sympathetic neurons. Journal of neurophysiology 82:2747-2764.

Lichtman JW, Purves D, Yip JW (1980) Innervation of sympathetic neurones in the guinea-pig thoracic chain. J Physiol 298:285-299.

McLachlan EM (2003) Transmission of signals through sympathetic ganglia--modulation, integration or simply distribution? Acta physiologica Scandinavica 177:227-235.

Percy WH, Krier J (1987) Cholinergic excitatory synaptic potentials of neurones in mammalian lumbar paravertebral ganglia. Journal of the autonomic nervous system 18:195-205.

Purves D, Lichtman JW (1985) Geometrical differences among homologous neurons in mammals. Science (New York, NY) 228:298-302.

Rimmer K, Horn JP (2010) Weak and straddling secondary nicotinic synapses can drive firing in rat sympathetic neurons and thereby contribute to ganglionic amplification. Frontiers in neurology 1:130.

Springer MG, Kullmann PH, Horn JP (2015) Virtual leak channels modulate firing dynamics and synaptic integration in rat sympathetic neurons: implications for ganglionic transmission in vivo. The Journal of physiology 593:803-823.

4) other achievements.

Difficulty in obtaining recordings from spinal cord injured tissue.

We've had considerable difficulty in obtaining access to the cellular properties of these neurons after spinal cord injury. One possibility is that the injury leads to the generation of novel structural/cellular components that surround sympathetic ganglia. The working hard at trying to modify experimental approach and have begun to obtain success in the last month. This data has yet to be analyzed. Having said that recording quality has still been suboptimal and we have just ordered dispase as an additional protease to apply in conjunction with collagenase in an attempt to make the neuronal tissue more accessible..

c. What opportunities for training and professional development has the project provided?

- *One individual was sent to a specialty meeting on spinal cord function in Marseille France to present his work and two individuals are being sent to the Annual Society for Neuroscience Meeting in San Diego this November.*

d. *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives*.

- *Continue investigations as proposed with much more focused effort on anatomical and cellular plasticity after spinal cord injury.*
- *We are now accumulated several ganglia for anatomical assessment but have yet to begun anatomical analysis.*

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Nothing to Report*
- **What was the impact on other disciplines?**
  - *Led to a CRCNS application with a computational neuroscientist.*
  - *Led to a R01 application with a computational neuroscientist*
- **What was the impact on technology transfer?**
  - *Nothing to Report*
- **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. L. MCKINNON, S. HOCHMAN. Patch clamp recordings of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia. Soc. Neurosci. Abst. 42 (2016).
2. Halder, M.C., M.; MacDowell, C.; McKinnon, M.; Sawchuk,M.; Hochman,S. (2016). Anatomy of mouse thoracic sympathetic chain ganglia and electrophysiological assessment of their multisegmental preganglionic input. Paper presented at: Society for Neuroscience.
3. Choi MHH (2015) Anatomical survey of paravertebral sympathetic chain in adult mice. In: Department of Neuroscience and Behavioral Biology: Emory.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

- *Mallika Halder – 25% effort – research specialist*
- *Michal McKinnon – 90% effort – graduate student*
- *Michael Sawchuk, - 50% 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?

- *PI. Craig H Neilsen Foundation. Continuous sensor-based home-cage recordings for SCI research. 10/16-10/19, $600,000 total.*
- *Co-PI. [Garraway PI] Craig H Neilsen Foundation. Compromised Aδ-LTMRs function contributes to allodynia after SCI 8/16-8/18, $300,000 total.*

f. What other organizations were involved as partners?

- *Nothing to Report*

8. SPECIAL REPORTING REQUIREMENTS

9. APPENDICES:

g. abstracts,

Session 302 - Network Interactions and Signal Propagation

● Add To Itinerary

302.18 / F19 - Anatomy of mouse thoracic sympathetic chain ganglia and electrophysiological assessment of their multisegmental preganglionic input

November 14, 2016, 8:00 - 12:00 PM Halls B-H

Presenter at Poster
Mon, Nov. 14, 2016, 9:00 AM - 10:00 AM

Session Type
Poster

Authors
***M. HALDER**[1], M. CHOI[2], C. MACDOWELL[2], M. MCKINNON[2], M. SAWCHUK[2], S. HOCHMAN[2];
[1]Emory Univ., Alpharetta, GA; [2]Emory Univ., Atlanta, GA

Disclosures
M. Halder: None. **M. Choi:** None. **C. MacDowell:** None. **M. McKinnon:** None. **M. Sawchuk:** None. **S. Hochman:** None.

Abstract
Paravertebral thoracic sympathetic postganglionic neurons (tSPNs) predominantly control vascular function in the upper/middle extremities yet their chain ganglia are practically inaccessible for physiological study. We developed an ex vivo adult mouse model that retains these chain ganglia in situ with intact ventral root (VR) connections to study pre- to post-ganglionic interconnections and undertook parallel anatomical studies to assess tSPN ganglia composition.We used thoracic VR stimulation to recruit segmental preganglionics to examine tSPN intersegmental population responses recorded with suction electrodes attached to individual ganglia. We observed multisegmental convergence of preganglionics onto individual thoracic ganglia. When recording from the T12 ganglia, we observed orthodromic responses after stimulation of T7-T12 VR. Overall, VR axons from the same segment usually evoked the largest responses in individual ganglia. Widespread divergence was also seen, particularly for mid-thoracic segments. For example, T6 VR stimulation evoked responses in all recorded ganglia (T2-T12). We examined the sensitivity of evoked synaptic responses to stimulus frequency and nicotinic acetylcholine receptor (nAChR) antagonists. Evoked responses were reproducible at 0.1 Hz but progressively depressed at 1 and 10 Hz. The common nAChR ganglionic blockers hexamethonium and mecamylamine depressed evoked responses consistent with actions on α3β4-containing nAChRs. Tubocurarine also depressed responses but DHβE did not. We used immunohistochemistry to determine the numbers and soma diameters of choline acetyltransferase$^+$ (ChAT$^+$) cholinergic and tyrosine hydroxylase$^+$ (TH$^+$) noradrenergic neurons in thoracic ganglia T1-T13 (n=2). Neuron numbers peaked at T7 and T8. TH$^+$ neurons comprised 97% of tSPNs which had slightly larger diameters than ChAT$^+$ neurons (17.5 vs. 16.7μm, respectively). There were more ChAT$^+$ tSPNs in segments rostral to T6. When counts of select ganglia from other mice were included, a remarkable inter-animal variability in ganglion neuron numbers was seen. We conclude that; (i) preganglionic convergence and divergence patterns onto tSPNs are abundant and multisegmental, (ii) evoked synaptic responses have nAChR pharmacology consistent with actions on α3β4-containing nAChRs, and (iii) tSPN ganglia have highly variable neuron numbers but are composed almost entirely of TH$^+$ adrenergic neurons.

Session 302 - Network Interactions and Signal Propagation

● Add To Itinerary

302.17 / F18 - Patch clamp recordings of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia

November 14, 2016, 8:00 - 12:00 PM Halls B-H

Presenter at Poster
Mon, Nov. 14, 2016, 8:00 AM - 9:00 AM

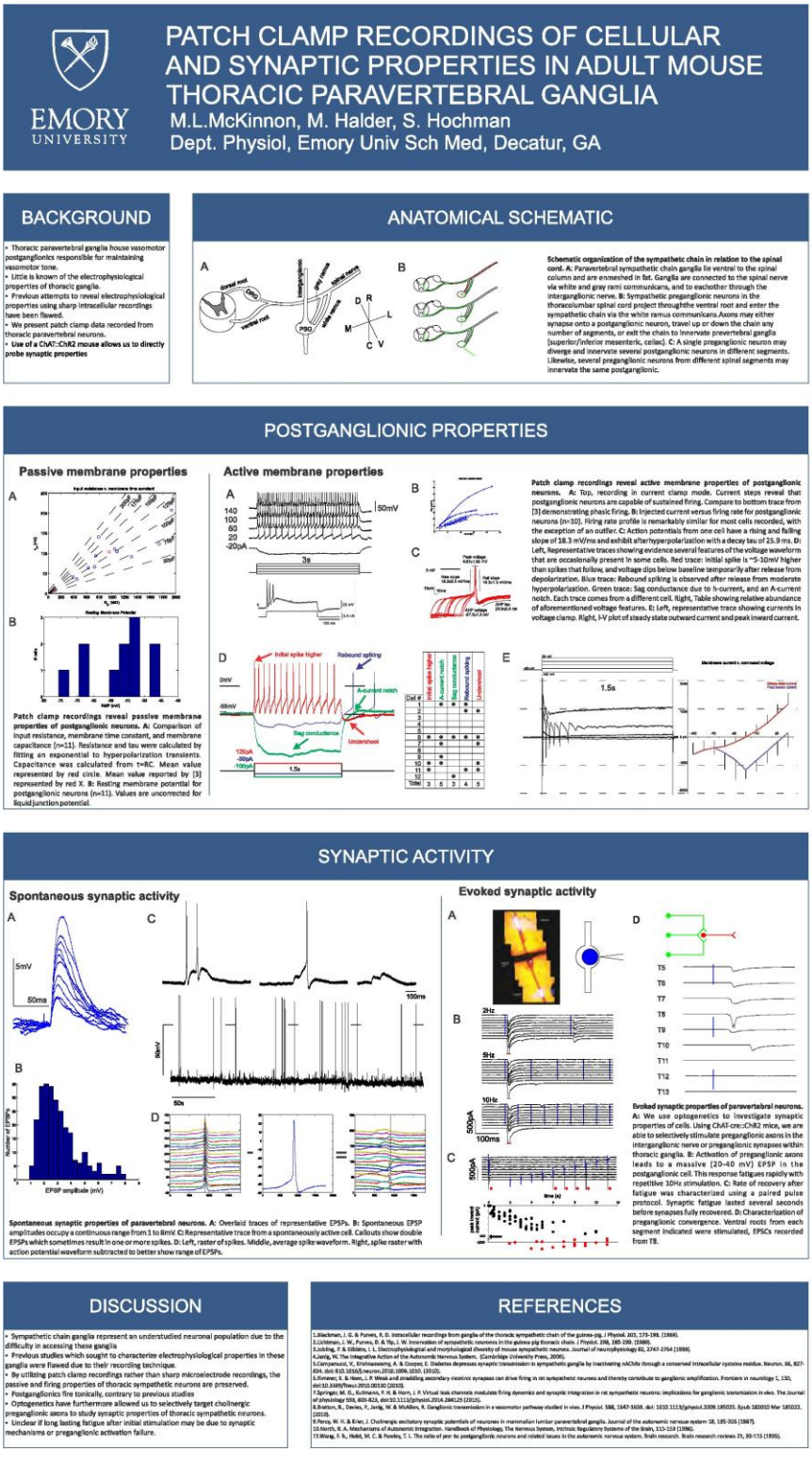
Session Type
Poster

Authors
***M. L. MCKINNON**, S. HOCHMAN;
Physiol., Emory Univ., Atlanta, GA

Disclosures
M.L. McKinnon: None. **S. Hochman:** None.

Abstract
Thoracic intraspinal preganglionic neurons project to sympathetic postganglionic neurons (SPNs) within paravertebral chain ganglia. While little is known of thoracic SPN functional properties, a major function is to control vasomotor tone. Paravertebral ganglia were traditionally thought to faithfully relay information from the spinal cord to the periphery, but this is an overly simplified viewpoint. To address this, we developed an ***in vitro*** approach for whole-cell recordings in intact thoracic ganglia (T3-T12) to characterize cellular and synaptic properties. Mean resting membrane potential was -57 mV. Average cell input resistance was 928 MΩ, and membrane time constant was 82 ms (n=8). These values are much higher than seen with sharp electrodes. A longer membrane time constant supports a greater temporal range for synaptic integration. In response to depolarizing current steps from rest, mean rheobase was 20 pA (n=3), and all exhibited a post-spike after-hyperpolarization. Some cells displayed post-inhibitory rebound spiking. While previous studies with sharp electrodes report phasic firing, we always observed sustained tonic firing and firing rate increased with increased current magnitude with a quasi-linear *f*-I relation. Sustained firing rates peaked at 17 Hz. Spontaneous EPSPs occupied a continuous range of amplitudes with a skewed distribution (mean=3.1mV, median=2.8mV). Several instances of temporal summation of EPSPs leading to spike recruitment were observed. We used ChAT::ChR2 mice to optogenetically recruit cholinergic axons to characterize synaptic responses. Blue light fiber-optic illumination typically elicited suprathreshold EPSPs and evoked responses fatigued dramatically with repeated stimulation. The rate of recovery after fatigue was characterized using a paired pulse protocol. Synaptic fatigue lasted several seconds before synapses fully recovered (n=5). We also characterized multisegmental convergence with optogenetic activation of preganglionic axons in ventral roots. Thoracic postganglionic neurons received presynaptic innervation from several contiguous spinal segments, and synaptic response amplitude varied with more proximal segments having a greater amplitude. In summary, thoracic SPNs have greater capacity for synaptic integration and spiking than previously considered, largely due to a reduced membrane leak, but recruitment may be restricted by severe synaptic fatigue observed at preganglionic cholinergic synapses. These data highlight the role of thoracic ganglia as active participants in vasomotor signal conditioning rather than passive relays of information.

h. posters


EMORY UNIVERSITY
PATCH CLAMP RECORDINGS OF CELLULAR AND SYNAPTIC PROPERTIES IN ADULT MOUSE THORACIC PARAVERTEBRAL GANGLIA
M.L.McKinnon, M. Halder, S. Hochman
Dept. Physiol, Emory Univ Sch Med, Decatur, GA
BACKGROUND
• Thoracic paravertebral ganglia house vasomotor postganglionics responsible for maintaining vasomotor tone.
• Little is known of the electrophysiological properties of thoracic ganglia.
• Previous attempts to reveal electrophysiological properties using sharp intracellular recordings have been flawed.
• We present patch clamp data recorded from thoracic paravertebral neurons.
• Use of a ChAT::ChR2 mouse allows us to directly probe synaptic properties
ANATOMICAL SCHEMATIC
Schematic organization of the sympathetic chain in relation to the spinal cord. A: Paravertebral sympathetic chain ganglia lie ventral to the spinal column and are enmeshed in fat. Ganglia are connected to the spinal nerve via white and gray rami communicans, and to eachother through the interganglionic nerve. B: Sympathetic preganglionic neurons in the thoracolumbar spinal cord project throughthe ventral root and enter the sympathetic chain via the white ramus communicans.Axons may either synapse onto a postganglionic neuron, travel up or down the chain any number of segments, or exit the chain to innervate prevertebral ganglia (superior/inferior mesenteric, celiac). C: A single preganglionic neuron may diverge and innervate several postganglionic neurons in different segments. Likewise, several preganglionic neurons from different spinal segments may innervate the same postganglionic.
POSTGANGLIONIC PROPERTIES
Passive membrane properties
Active membrane properties
Patch clamp recordings reveal passive membrane properties of postganglionic neurons. A: Comparison of input resistance, membrane time constant, and membrane capacitance (n=11). Resistance and tau were calculated by fitting an exponential to hyperpolarization transients. Capacitance was calculated from τ=RC. Mean value represented by red circle. Mean value reported by [3] represented by red X. B: Resting membrane potential for postganglionic neurons (n=11). Values are uncorrected for liquid junction potential.
Patch clamp recordings reveal active membrane properties of postganglionic neurons. A: Top, recording in current clamp mode. Current steps reveal that postganglionic neurons are capable of sustained firing. Compare to bottom trace from [3] demonstrating phasic firing. B: Injected current versus firing rate for postganglionic neurons (n=10). Firing rate profile is remarkably similar for most cells recorded, with the exception of an outlier. C: Action potentials from one cell have a rising and falling slope of 18.3 mV/ms and exhibit afterhyperpolarization with a decay tau of 25.9 ms. D: Left, Representative traces showing evidence several features of the voltage waveform that are occasionally present in some cells. Red trace: Initial spike is ~5-10mV higher than spikes that follow, and voltage dips below baseline temporarily after release from depolarization. Blue trace: Rebound spiking is observed after release from moderate hyperpolarization. Green trace: Sag conductance due to h-current, and an A-current notch. Each trace comes from a different cell. Right, Table showing relative abundance of aforementioned voltage features. E: Left, representative trace showing currents in voltage clamp. Right, I-V plot of steady state outward current and peak inward current.
SYNAPTIC ACTIVITY
Spontaneous synaptic activity
Evoked synaptic activity
Spontaneous synaptic properties of paravertebral neurons. A: Overlaid traces of representative EPSPs. B: Spontaneous EPSP amplitudes occupy a continuous range from 1 to 8mV. C: Representative trace from a spontaneously active cell. Callouts show double EPSPs which sometimes result in one or more spikes. D: Left, raster of spikes. Middle, average spike waveform. Right, spike raster with action potential waveform subtracted to better show range of EPSPs.
Evoked synaptic properties of paravertebral neurons. A: We use optogenetics to investigate synaptic properties of cells. Using ChAT-cre::ChR2 mice, we are able to selectively stimulate preganglionic axons in the interganglionic nerve or preganglionic synapses within thoracic ganglia. B: Activation of preganglionic axons leads to a massive (20-40 mV) EPSP in the postganglionic cell. This response fatigues rapidly with repetitive 10Hz stimulation. C: Rate of recovery after fatigue was characterized using a paired pulse protocol. Synaptic fatigue lasted several seconds before synapses fully recovered. D: Characterization of preganglionic convergence. Ventral roots from each segment indicated were stimulated, EPSCs recorded from T8.
DISCUSSION
• Sympathetic chain ganglia represent an understudied neuronal population due to the difficulty in accessing these ganglia
• Previous studies which sought to characterize electrophysiological properties in these ganglia were flawed due to their recording technique.
• By utilizing patch clamp recordings rather than sharp microelectrode recordings, the passive and firing properties of thoracic sympathetic neurons are preserved.
• Postganglionics fire tonically, contrary to previous studies
• Optogenetics have furthermore allowed us to selectively target cholinergic preganglionic axons to study synaptic properties of thoracic sympathetic neurons.
• Unclear if long lasting fatigue after initial stimulation may be due to synaptic mechanisms or preganglionic activation failure.
REFERENCES