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

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Fort Detrick, Maryland 21702-5012

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

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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?
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.

<table>
<thead>
<tr>
<th>Characterizing thoracic chain sympathetic postganglionics</th>
<th>months</th>
<th>% completion/ Completion dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major Task 1a: Convergence and divergence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtask 1: Segment specific properties</td>
<td>1-6</td>
<td>75%</td>
</tr>
<tr>
<td>Subtask 2: Pharmacology</td>
<td>7-12</td>
<td>75%</td>
</tr>
<tr>
<td>Subtask 3: Breeding/crossing transgenic mice and spinalizations</td>
<td>1-36</td>
<td>18 months behind target</td>
</tr>
<tr>
<td>Subtask 3: Establish intracellular recording techniques</td>
<td>3-18</td>
<td>100%</td>
</tr>
</tbody>
</table>

| **Major Task 1b: Convergence and divergence**             |        |                                |
| 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

<table>
<thead>
<tr>
<th>Intracellular recordings and optogenetics</th>
<th>months</th>
<th>% completion/ Completion dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major Task 2: Characterize mechanisms responsible for dysautonomia after spinal cord injury using intracellular recordings and optogenetics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtask 1: Physiological plasticity in preganglionic-postganglionic interactions assessed using optogenetics</td>
<td>18-36</td>
<td>20%</td>
</tr>
<tr>
<td>Subtask 2: Physiological plasticity in afferent-postganglionic interactions assessed using optogenetics</td>
<td>18-36</td>
<td>0%</td>
</tr>
<tr>
<td>Subtask 3: Physiological plasticity in preganglionic-afferent interactions assessed using optogenetics</td>
<td>18-36</td>
<td>0%</td>
</tr>
<tr>
<td>Subtask 4: Intracellular recordings of synaptic and cellular plasticity in membrane properties; demonstration of membrane bistability</td>
<td>18-36</td>
<td>25%</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Data analysis and publications</th>
<th>months</th>
<th>% completion/ Completion dates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major Task 3: Data analysis and publications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtask 1: Data analysis</td>
<td>6-36</td>
<td>55%</td>
</tr>
<tr>
<td>Subtask 2: Manuscript writing and submission</td>
<td>24-36</td>
<td>40%</td>
</tr>
</tbody>
</table>

**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 Sylgaard recording chamber and suction electrodes are positioned to stimulate various thoracic ventral roots and record from various thoracic ganglia.

Progress/results: In the annual progress report in 2016 we used extracellular recordings to show that there is a convergence onto individual ganglia. For example, stimulating T4-T11 ventral roots results in activity in the T11 ganglion. The studies involved electrical stimulation of ventral roots and we proposed to repeat these trials using a genetic approaches for optical stimulation of ventral roots. The advantage here is that recruitment is likely in size principle order and that use of ChAT::CHR2 ensures that axonal recruitment from ventral roots is exclusively recruiting preganglionic cholinergic neurons and not inadvertently activating primary afferents that we showed previously and as has been reported also project visceral afferents through some ventral roots in thoracic segments. We have just begun assessment using optogenetics including after spinal cord injury.

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. We have increase sample size in the previous year and we’ve also broadened our pharmacological approach to include assessment of neuromodulation by sympathomimetics that include octopamine as well as β-phenylethylamine. Intracellular Recordings. Experiments continue to assess the effects of various channel blockers on intrinsic membrane currents and synaptic events in intracellular recordings from individual neurons (Figure 1).

Figure 1. tSPN spontaneous EPSPs are cholinergic and modulated by PEA. A. Overlaid traces of captured EPSPs in one spontaneously active cell. B. Histogram of events showing that EPSP amplitudes occupy a continuous range from 1 to 8mV. C-D. Spontaneous EPSPs are cholinergic. They were blocked by 100μM hexamethonium (nAChR antagonist), and enhanced by 10μM neostigmine (acetylcholinesterase). E. 30μM PEA dramatically increased the amplitude and frequency of spontaneous EPSPs. Blue arrow: spontaneous EPSP. Red arrow: spontaneous EPSP triggered spike.
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 transgensics 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.
As stated in the annual progress report in 2016, spinalizations are behind schedule. This has not changed. For example, in our last series of spinalizations with n=5, only one survived the requisite three week period we deemed necessary to examine plasticity at a time with known autonomic dysreflexia. Two animals were sacrificed early after spinalization (in the first week) due to health concerns. Two mice died from ruptured bladders due to manual expression even though individuals undertaking manual expression has significant experience, it appears that the bladder itself becomes more easily ruptured with manual expression pressures that previously were not sufficient to induce rupture. 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.

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 (and now dispase) 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 now fully achieve acceptable recordings from most mice used in experiments, with recordings that can last > 1 hour. These longer recordings are required to characterize convergent synaptic input properties and to study membrane current pharmacology. 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. Details were described in the annual progress report for 2016. We have now begun to examine these evoked responses after SCI in the data has yet to be fully analyzed. Please refer back to last year’s annual report for detailed observations.

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: & Progress/results: We have abandoned these experiments due to unanticipated difficulty in success rates and other experiments as well as difficulty in maintaining our Advillin-Cre breeding population.

2.3: Physiological plasticity in preganglionic-afferent interactions assessed using optogenetics
Methods/experiment: & Progress/results: We have abandoned these experiments due to unanticipated difficulty in success rates and other experiments as well as difficulty in maintaining our Advillin-Cre breeding population.

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=8, 3 of which are at early injury time, 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 and results sections are essentially complete. The results section is still in progress.

Further UPDATES.

(A) Characterization of cellular properties in adult mouse thoracic paravertebral ganglia.

By using whole-cell patch clamp recordings in intact thoracic ganglia, we have been able to record tSPNs in intact ex vivo thoracic ganglia to characterize their cellular and synaptic properties. We now have a large dataset of 39 healthy cells is shown in Table 2 (mean values ± SD). Resting membrane potential, input resistance and membrane time constant (\(T_m\)) were substantially higher than those reported in previous studies in the adult mouse (resting membrane potential is 10 mV lower, input resistance is 9 times higher and \(T_m\) is 13 times longer) (Jobling and Gibbins, 1999). Rheobase varied greatly between cells, but values were still approximately 10 times lower than those reported previously (Jobling and Gibbins, 1999). Threshold voltage was typically 18 mV higher than resting membrane potential, and action potentials displayed after-hyperpolarization. All neurons were capable of repetitive firing, in contrast to previous reports of only phasic firing with depolarizing current (Jobling and Gibbins, 1999). These differences are most likely due to the preservation of cell physiology with our whole-cell patch in contrast to the disruption of cell properties by impalement injury using sharp electrodes in previous studies. In our whole-cell patch, maximal firing rates observed in response to depolarizing current steps ranged from 14-17 spikes/sec. During intracellular depolarization, firing rate increased with increased current injection and cells sustained tonic firing. Spike frequency adaptation was also observed. All recorded properties are fully consistent with those reported recently with whole cell recordings in the rat superior cervical ganglia (Springer et al., 2015). We also observed a notable \(I_h\) current in 8 out of 13 cells. Its activation generally required hyperpolarization beyond -100 mV and \(I_h\) current was more pronounced with greater hyperpolarization. With activation of \(I_h\) current, cells often displayed a post-inhibitory rebound spike, which may be a major factor contributing to oscillatory activity discussed below. We are also able to gauge the magnitude of A-type potassium currents (\(I_A\)). The current amplitude of \(I_A\) current amplitude following a hyperpolarization voltage step is comparable to reported study is comparable, but of much longer duration when compared to prior reports.

While a full manuscript for submission on these membrane properties was expected to be submitted by June, additional observations and incorporation of additional modeling has extended the process and we now anticipate a submission date of December 2017. The current version of the manuscript is attached.

Comparing cellular properties after SCI.

Changes in connectivity following SCI may involve anatomical changes in tSPNs themselves. First, in the sparsely-labeled TH::tdTomato healthy animal, we observed very few dendrites in adrenergic neurons in caudal compared to rostral thoracic paravertebral ganglia (annual report 2016). This lack of dendrites in caudal

Table 2. Summary of basic membrane properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Mean ± SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane properties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>-58.8 ± 7.2 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Input Resistance, MΩ</td>
<td>1072 ± 553 (38)</td>
<td>38</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>94.3 ± 54.8 (38)</td>
<td>38</td>
</tr>
<tr>
<td>Capacitance, pF</td>
<td>89.2 ± 26.8 (38)</td>
<td>38</td>
</tr>
<tr>
<td>Threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute voltage, mV</td>
<td>-41.2 ± 7.1 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Relative to (V_{rest}), mV</td>
<td>26.0 ± 7.7 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Rheobase, pA</td>
<td>27.5 ± 16.0 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Action Potential</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>55.0 ± 15.7 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Peak, mV</td>
<td>13.8 ± 18.2 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Half-width, ms</td>
<td>4.6 ± 1.1 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Rise slope, mv/ms</td>
<td>47.3 ± 24.2 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Afterhyperpolarization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>15.1 ± 3.7 (26)</td>
<td>26</td>
</tr>
<tr>
<td>Half-decay, ms</td>
<td>80.8 ± 34.9 (26)</td>
<td>26</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>230 ± 71 (26)</td>
<td>26</td>
</tr>
<tr>
<td>F-I slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max., Hz/pA</td>
<td>0.126 ± 0.033 (39)</td>
<td>39</td>
</tr>
<tr>
<td>Sustained, Hz/pA</td>
<td>0.075 ± 0.025 (39)</td>
<td>39</td>
</tr>
</tbody>
</table>
ganglia is an important factor in considerations of tSPN excitability, including lack of persistent inward currents (PICs) and membrane bistability. In motoneurons, membrane bistability is associated with dendritic expression of PIC related voltage-gated channels. Thus anatomical changes such as increased dendritic arborization of tSPN will be consistent with the hypothesis that PICs emerge post-SCI.

Preliminary recordings suggest that $I_A$ activation/inactivation dynamics may be lengthened after SCI (Figure 2). These preliminary studies of intrinsic cellular properties of unidentified tSPNs provide a demonstration of the power of whole patch recordings for discovery of tSPN physiology. With specific targeting of NPY-positive vasoconstrictor tSPNs, I will be able to definitively determine intrinsic properties related to vasomotor function.

(B) Anatomical and synaptic plasticity after spinal cord injury.

Anatomical plasticity after spinal cord injury.

We have now compared counts and diameters of thoracic sympathetic postganglionic neurons from the T5 segment. Samples were in naïve controls ($n=5$) and mice having undergone spinal transection at thoracic level two (T2) three weeks prior ($n=7$). Adrenergic neurons were identified in whole ganglion immunohistochemical reaction for tyrosine hydroxylase (TH). Counts and size (area/diameter) of T5 neurons positive for TH were undertaken using Neurolucida software (MicroBrightField). We conducted t-tests with a significance level of $\alpha=0.05$. We observed that after SCI, mean area and diameter of adrenergic neurons were statistically decreased (Table 1). We also compared average cell numbers, though there was a numerical 28% reduction in cell numbers after SCI, the observed significant variability and low sample size did not provide sufficient power to reliably determine statistical significance. Future plans are to increase our sample size as well as extend observations to other ganglia.

Significant differences in cell area and diameter between SCI and naive T5 ganglia could be due to influence of sex rather than treatment. However, when we compared the average area and diameter of male versus females we saw no significant differences in mean areas or diameters. Within the constraints of our limited population size, we conclude that sex is not a factor.

Synaptic properties of paravertebral neurons.

The previous annual report (2016) provided details of our recordings of spontaneous and optogenetically evoked synaptic responses. This past year was associated with breeding issues that prevented us from undertaking various optogenetic stimulation experiments. Nonetheless we have had good success with increasing our success rate of whole cell recordings and this will enable a more complete assessment of ongoing spontaneous synaptic activity in the naïve preparation.

We have just begun to assemble data set of evoked responses in the spinal cord injured animals, but the data is too recent to provide quantitative analyses and is simply shown in figure that we believe is representative of observed differences (Figure 3).

We have also just begun to use an optogenetic approach to assess divergence of preganglionic axons arising from spinal segments onto individual thoracic chain ganglia onto individual tSPNs (Fig X). These results are also very recently obtained and preclude position of quantitative assessment at this stage.
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.

We have found this to make an enormous difference and now have recordings from several neurons after spinal cord injury.

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.
Three undergraduate students have worked on this project. Two of them I have worked on this model system in the last year, with one student undertaking a senior research project with poster present (attached).

d. Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.
  o We have received a no-cost extension, we plan to stay consistent with the major tasks outlined in the charts except for Major Task 2, subtasks 2 and 3.
  o Regarding electrophysiology, emphasis will be on assessment of physiological plasticity
  o Regarding anatomical assessment, we will continue towards the changed emphasis on more overtly describing the previously implicit neuroanatomical assessment of injury-induced plasticity using immunolabeling approaches.
  o During this no-cost extension, a significant amount of time will be devoted to data analysis and manuscript writing.

4. IMPACT:

  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.
    ▪ Led to a R01 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:

Please see above. We have a no-cost extension to try and complete some of the major goals of the grant.

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.

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
- Yaqing Li – 33% effort – postdoctoral fellow
- Lucy Galvin – 10% effort – Senior undergraduate research project

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?

- P.I. NIH R01. Recruitment principles and injury-induced plasticity in thoracic paravertebral sympathetic postganglionic neurons. 6/2017-6/2022, $1,250,000 direct.
- 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. paper in preparation

Introduction (500 words)

Mammals are a highly successful mammalian group with the largest number of species. In this study, we investigated the influence of maternal stress on postnatal behavioral development in a comparative study across two species, the laboratory mouse (Mus musculus) and the red-eared slider turtle (Trachemys scripta elegans). We divided the animals into two groups: control and stress. The control group received standard laboratory conditions, while the stress group was subjected to chronic social stress. Behavioral tests were conducted at different stages of development, including postnatal periods 1, 2, and 3. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Significance statement (120 words)

The influence of maternal stress on postnatal behavioral development is crucial for understanding the impact of early life experiences on the development of emotional and social behaviors. Chronic social stress has been shown to affect anxious-like, locomotor, and social behaviors in both laboratory mice and red-eared slider turtles. These findings suggest that stress has a profound effect on the development of emotional and social behaviors in both species. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development. The results of this study contribute to the understanding of the effects of stress on the development of emotional and social behaviors in comparative studies.

Methods

The control group received standard laboratory conditions, while the stress group was subjected to chronic social stress. Behavioral tests were conducted at different stages of development, including postnatal periods 1, 2, and 3. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Figures

Figure 1: Schematic diagram showing the experimental design of the study. The control group received standard laboratory conditions, while the stress group was subjected to chronic social stress. Behavioral tests were conducted at different stages of development, including postnatal periods 1, 2, and 3. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Figure 2: Graph showing the changes in anxious-like behaviors in the control and stress groups across the different postnatal periods. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Figure 3: Graph showing the changes in locomotor behaviors in the control and stress groups across the different postnatal periods. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Figure 4: Graph showing the changes in social behaviors in the control and stress groups across the different postnatal periods. The results showed that chronic social stress had a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors. These findings suggest that stress has a profound effect on the development of social and emotional behavior in both species. The data highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development.

Discussion

The results of this study suggest that chronic social stress has a significant impact on behavioral development, affecting anxious-like, locomotor, and social behaviors in both laboratory mice and red-eared slider turtles. These findings highlight the importance of considering the effects of stress on the development of emotional and social behaviors in comparative studies. The implications of these findings are discussed in the context of understanding the neurobiological mechanisms underlying stress and behavioral development. The results of this study contribute to the understanding of the effects of stress on the development of emotional and social behaviors in comparative studies.

Data availability

All data generated or analyzed during this study are included in the published article. The data will be made available upon request from the corresponding author.
Characterization of cell number and size in T5 paravertebral ganglia following T2 spinal cord transection: Implications for autonomic dysreflexia.

Galvin ML, Sokoloff AJ, Sawchuk M, Hochman S, Emory University, Department of Physiology

INTRODUCTION

Autonomic dysreflexia (AD) is a potentially life-threatening condition that results from spinal cord injury (SCI) above the T5 spinal level. The condition is especially severe in patients with an incomplete lesion involving the thoracic sympathetic ganglia. AD is considered a medical emergency requiring immediate attention because persistent elevated hypertension can lead to stroke or death.

AD is initiated by a noxious stimulus arising below the injury level, typically bladder distention, bowel urgency, or bowel evacuation. In a healthy individual, sympathetic vasoconstrictor reflexes are activated in response to noxious stimuli in the periphery to protect vital organs. However, in SCI patients, ascending sensory signals are blocked at the site of the injury and prevent perception of noxious signals. Additionally, disruption of descending control of the sympathetic nervous system due to SCI prevents a normal physiological response to decrease hypertension. In this situation, noxious afferents have complete control over activation of sympathetic neurons resulting in a large and persistent increase in blood pressure (Figure 1).

METHODS AND ANALYSIS

METHODS

- Mouse model: male and female
- Spinal transection at thoracic level two (T2)
- TCI/Carex, TS control
- Twenty-one day survival
- Harvest of T5 ganglia
- Whole ganglia immunohistochemical reaction for tyrosine hydroxylase (TH)
- Count and size (area/diameter) of T3 neurons positive for TH in the neuropeptide software

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RESULTS

Area

The difference in the mean values of the average area for SCI versus TS is greater than would be expected by chance. There is a statistically significant difference between the treatment groups’ average cell area (F = 0.028). At an alpha level of 0.05, we can deduce that this difference in means is statistically significant. However, the power of the performed test (0.019) is below the desired power of 0.80. Less than desired power indicates we are less likely to detect a difference when one actually exists.

Diameter

The difference in the mean values of the diameter for SCI versus TS is greater than would be expected by chance. There is a statistically significant difference between the treatment groups (F = 0.028). At an alpha level of 0.05, we can deduce that this difference in means is statistically significant. The power of the performed test (0.019) is below the desired power of 0.80. Less than desired power indicates we are less likely to detect a difference when one actually exists.

We hypothesized that the statistically significant differences in cell area and diameter between SCI and TS T5 ganglia could be due to the influence of sex rather than treatment. However, when we compared the average area and diameter of male versus female SCI we saw no significant differences in mean areas or diameters. Within the constraints of our limited population size, we conclude that sex is not a factor.

DISCUSSION

We hypothesize that, in response to the exaggerated sympathetic response associated with the amplified sensory input, male animals are more likely to develop a greater degree of autonomic dysreflexia compared to female animals. This hypothesis is supported by the observed differences in cell area and diameter between SCI and TS T5 ganglia.

Further studies would have to control for confounding factors such as sex, left or right ganglia. In addition, we would have to collect more data from more animals in order to increase statistical power.

Acknowledgements

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References


h. posters