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TITLE: Determining Sensory Plasticity and Developing Recovery for Sexual Dysfunction in Chronic Spinal Cord-Injured Male Rats

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CONTRACTING ORGANIZATION: Kent State University, Kent, OH

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In the current proposal, the hypothesis will be tested that chronic SCI causes long term deficits in transmission of sensory inputs related to sexual activity to the spinal ejaculation generator. It is hypothesized that chronic spinal injury causes reorganization of the sensory fibers that relay sensory information from the sexual organs to the spinal ejaculation generator. In the first aim, tract tracing injections are utilized to identify changes in C and Aδ fibers in the main sensory nerve that relays sensory information related to sexual activity. Significant progress has been made towards this aim and further analysis is ongoing. Secondly, the hypothesis is tested that chronic contusion injury alters glutamate inputs to and expression of glutamate receptors in the spinal ejaculation generator. Confocal microscopy analysis was used to investigate changes in glutamatergic inputs to spinal neurons in the ejaculation generator using immunolabeling for vesicular glutamate transporters 1 and 2 and galanin for LSt cells. Indeed, it was demonstrated that chronic contusion injury caused a significant reduction of glutamatergic axon inputs to the spinal ejaculation generator, which may in turn contribute to ejaculatory dysfunction following spinal cord injury. Finally, studies will aim to test effectiveness of glutamate receptor agonists on recovery and restoration of ejaculatory function following spinal cord injury.

15. SUBJECT TERMS
None listed.

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1. Introduction

Surveys of men with spinal cord injury (SCI) have demonstrated that among paraplegic men, regaining sexual function is the most important goal and will significantly improve quality of life. Hence, the development of novel, innovative interventions is of significant relevance for patients in both the military and civilian populations. Ejaculatory dysfunction in particular is extremely common among SCI men and less than 10% of men with SCI can ejaculate without the aid of medical interventions. Despite the identified need and desire to improve sexual function among SCI men, the underlying mechanisms of this key health problem have been largely unexplored, and we have a poor understanding of the mechanisms by which chronic injury so radically influences the spinal ejaculation generator. Several years ago, our laboratory identified the pivotal cell population that comprises the spinal ejaculation generator in male rats and has since made great strides towards a better understanding of this spinal ejaculation generator. Moreover, we recently demonstrated that chronic SCI caused long-term deficits of ejaculatory function in male rats, using a contusion injury paradigm that closely resembles the neural trauma in human spinal cord injury. In the current proposal, we will test the hypothesis that chronic SCI causes long term deficits in the transmission of sensory inputs related to sexual activity to the spinal ejaculation generator in the male rat. We will test specific hypotheses that SCI causes remodeling of the sensory nerves and expression of the neural transmitters and peptides in these nerves. Moreover, we will conduct experiments using pharmacological intervention with the goal to restore ejaculatory dysfunction following chronic SCI. Together, these studies will form a first critical step towards identifying SCI-induced deficits in sensory inputs that control ejaculation and will form the basis for the development of treatment strategies.

2. Keywords

Spinal cord injury, sexual dysfunction, anejaculation, plasticity, sensory, peripheral nerve

3. Accomplishments

Major Goals of the Project:
Major Task 1: Study Regulatory Set-up
Major Task 2 (Specific Aim 1): To test the hypothesis that chronic spinal injury caused a reorganization of the sensory fibers that relay sensory information from the sexual organs to the dorsal horn of the lumbosacral spinal cord and the LSt cells.
Major Task 3 (Specific Aim 2): To test the hypothesis that chronic SCI disrupts processing of sensory inputs by ablating glutamate inputs to LSt cells.

Accomplishments under these goals:
Major Task 1: Study Regulatory Set-up:
Subtask 1.1: Submit documents to the Kent State University IACUC and obtain approval.
Progress: Completed in prior budget periods.

Subtask 1.2: Submit animal protocol to DoD's ACURO and make revisions as necessary.
Progress: Completed in prior budget periods.

Subtask 1.3 and 1.4: Hire and train study personnel.
Progress: Completed in prior budget periods.

Major Task 2 (Specific Aim 1): To determine whether chronic spinal injury caused a reorganization of the sensory fibers that relay sensory information from the sexual organs to the dorsal horn of the lumbosacral spinal cord and the LSt cells.
Subtask 2.1: Aim 1A: To determine if chronic SCI increases C-fibers in the dorsal penile nerve (DPN) using tract tracing.
Subtask 2.2: Aim 1B: To test if SCI increases neuropeptide expression in C-fibers of DPN.
Subtask 2.3: Publications Aim 1A and 1B

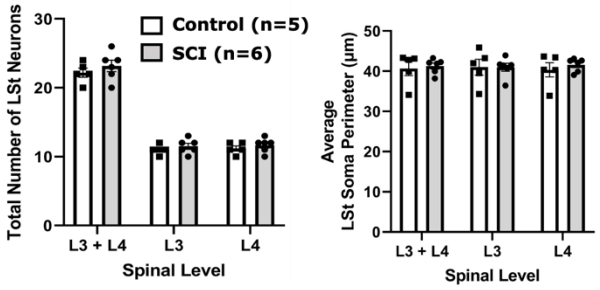
Final progress: Despite numerous attempts during prior budget years, we were unable to complete this aim in a reliable, reproducible, and rigorous manner. As reported in previous annual and quarterly reports, tract tracing injections resulted in variable results and efforts to delineate C-fibers were unsuccessful using the proposed approaches. We also tried additional approaches not included in the original proposal, including electron microscopy, but none of these experiments yielded rigorous and reproducible results. Thus, even though some progress was made towards Aim 1 in the first years of the award, these data weren't sufficiently rigorous and reliable and won't be published. This question thus remains outstanding and relevant.

Major Task 3 (Specific Aim 2): To test the hypothesis that chronic SCI disrupts processing of sensory inputs by ablating glutamate inputs to LSt cells.
Subtask 3.1 (Aim 2A): To determine if SCI alters LSt cells responsiveness to glutamate by reducing glutamate inputs to LSt cells and glutamate receptor expression in LSt cells.

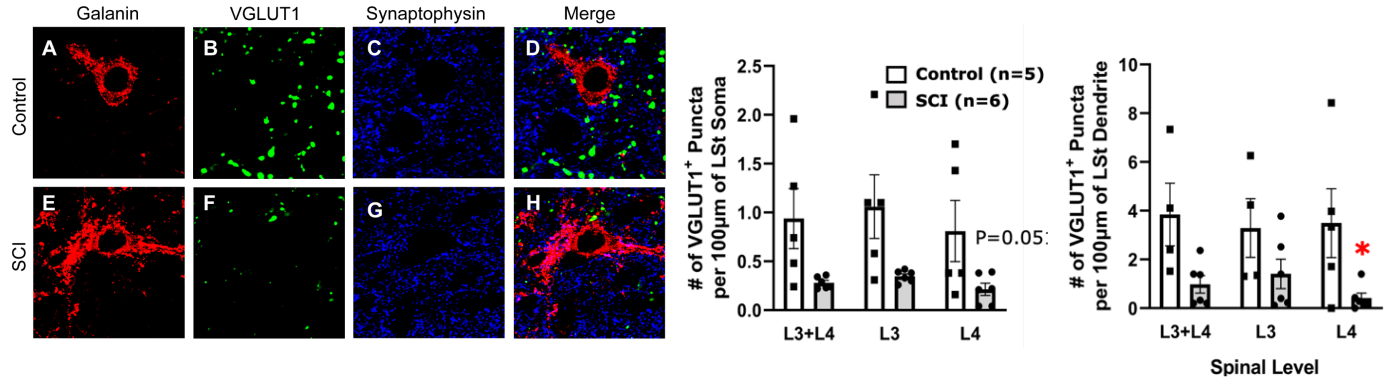
Final Progress: This aim has been completed and numerous findings will be reported for this aim. During the final reporting period, findings from previous budget years were re-analyzed, reproduced, and analyzed for final presentation and visualization of data. In addition, new data were generated, and all have been described in a manuscript currently in final stage of preparation for submission.

Summary of main results and conclusions:

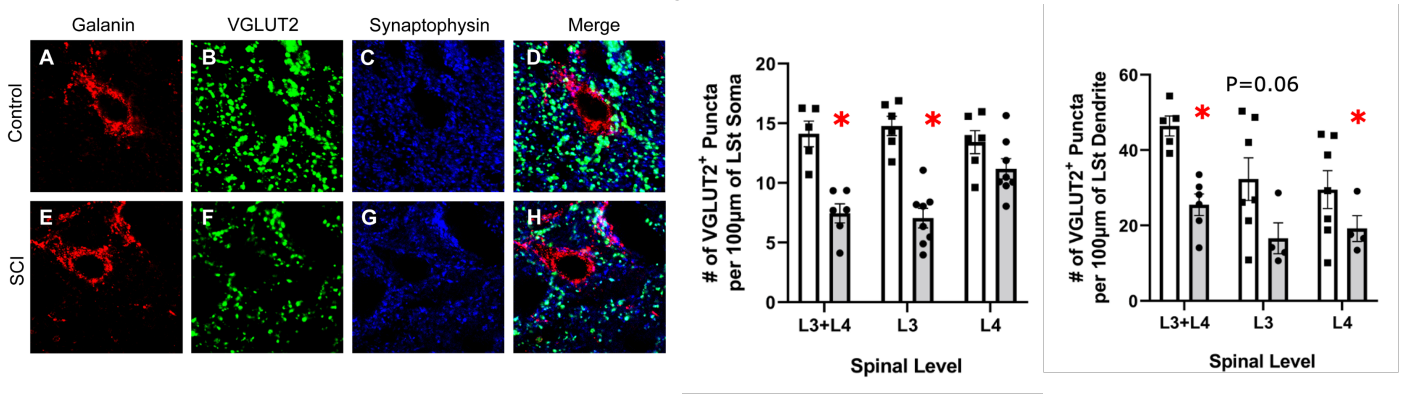
1. SCI didn't alter the number or soma size of LSt neurons (4 weeks after injury), replicating our previous findings.



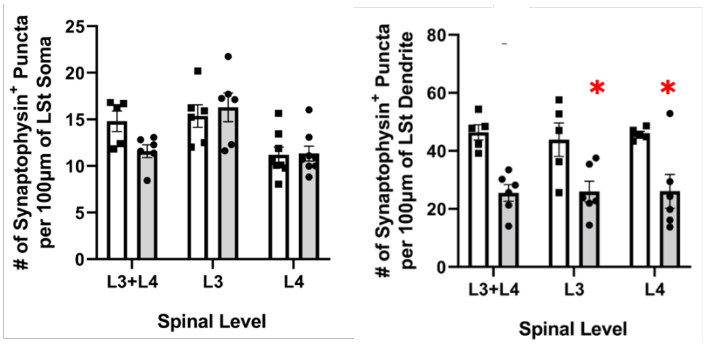
2. Immunofluorescent staining and confocal analysis demonstrated that VGLUT1^{+} synapses sparsely innervate LSt soma and dendrites, and were reduced following SCI (4 weeks after injury).



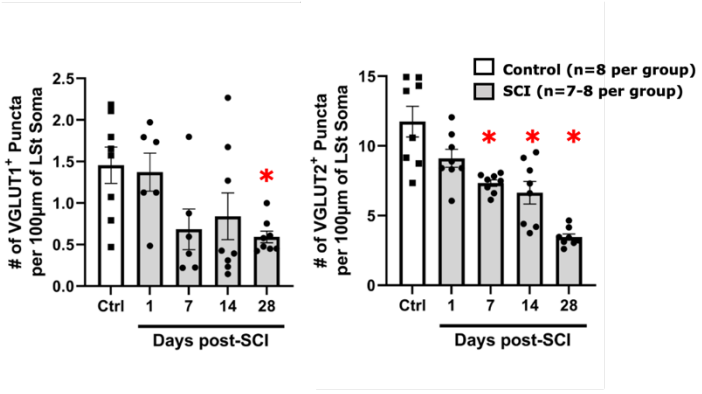
3. Immunofluorescent staining and confocal analysis demonstrated that VGLUT2^{+} synapses richly innervate LSt soma and dendrites, and were reduced following SCI.



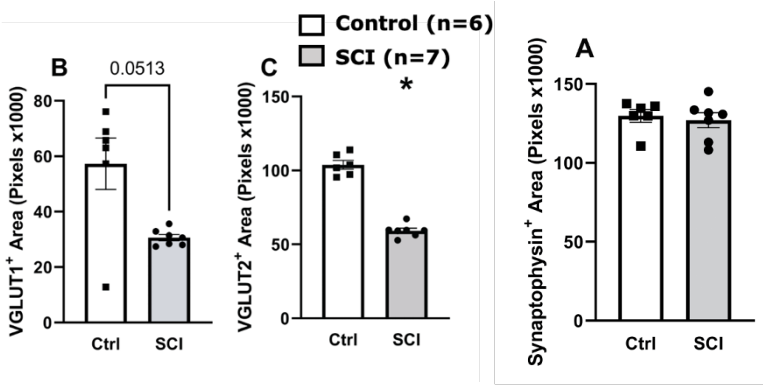
4. Immunofluorescent staining and confocal analysis demonstrated that SCI also reduced other synaptic inputs to LSt dendrites, but not LSt soma.



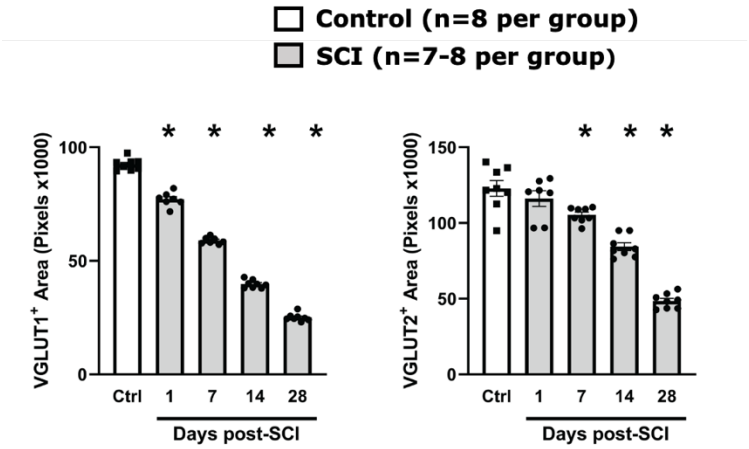
5. A time course study demonstrated that VGLUT1^{+} and VGLUT2^{+} synapse loss onto LSt neurons was time dependent.



6. Densitometry analysis demonstrated that glutamatergic synapse loss was not limited to axons contacting LSt neurons and instead occurred generally in laminae VII and X (4 weeks after injury):
    a. SCI reduced VGLUT2 puncta in laminae VII and X (statistical trend for VGlut1).
    b. SCI did not reduce overall synaptophysin in laminae VII and X.

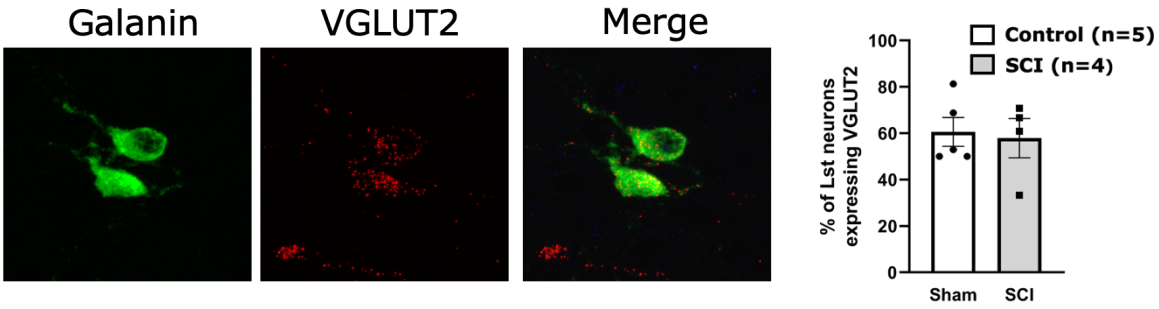


    c. Loss of VGLUT1 and VGLUT2 puncta in in laminae VII and X was time dependent.



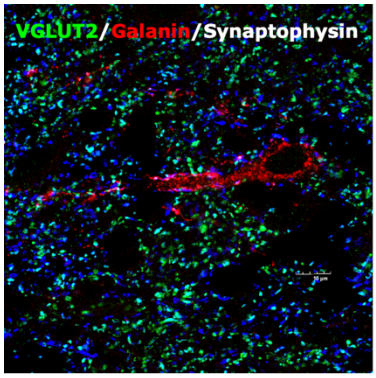
7. Analysis of VGLUT1 and VGLUT2 mRNA expression in the lumbosacral spinal cord using fluorescent In Situ Hybridization using RNAscope revealed that SCI didn't affect expression of VGLUT2 in laminae VII and X, not in laminae III and IV or laminae I and II at any of the lumbar spinal levels. VGLUT1 was not expressed in interneurons in lumbar spinal spinal cord. Thus, these results suggested that SCI-induced loss of glutamatergic axons was not caused by cell death or a loss of transcript in glutamatergic interneurons, and is likely associated with reduced translation of transport of VGLUT2 protein.

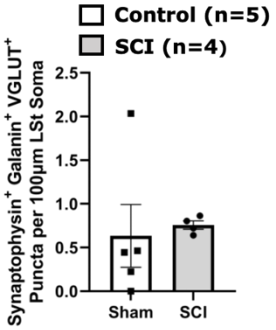
8. The RNAScope analysis demonstrated that *VGLUT2* mRNA (not VGLUT1) is expressed by LSt cells. Immunolabeling studies thus far had not detected any co-labeling of VGLUT2 and galanin, so the finding of transcript in LSt cells was unexpected. However, *VGLUT2* mRNA levels in LSt cells were not altered by SCI.



9. To again confirm that VGlut2 immunoreactivity was not present in LSt cells, further immunolabeling was conducted using different antibodies and staining protocols. But again, it was demonstrated that VGLUT2-

immunoreactivity was detected in very few (if any) galanin axons, showing that there were very few VGLUT2^{+} LSt to LSt synapses, and these were not altered by SCI.





In conclusion, these studies demonstrated that:

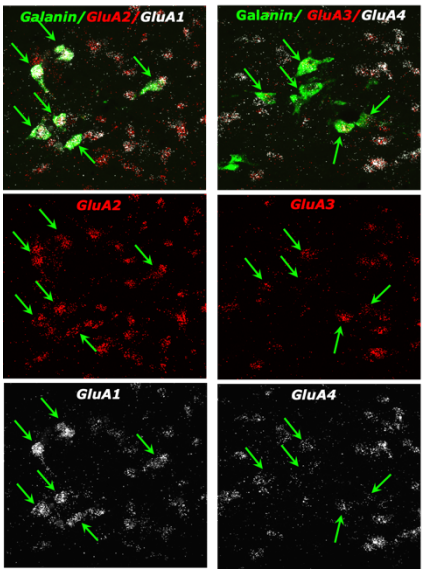
- Chronic SCI resulted in loss of glutamatergic synapses onto LSt neurons of the spinal ejaculation generator.
- The bulk of excitatory input onto LSt neurons contains VGLUT2, with fewer VGLUT1 terminals, both of which are reduced by chronic SCI.
- LSt cells express *VGLUT2* mRNA but are not the local source of VGLUT2 inputs to LSt cells.
- Glutamatergic synapse loss progresses over time, suggesting long term alterations and plasticity.
- Glutamatergic synapse loss is not limited to LSt neurons and occurs in the proximity of LSt cells.

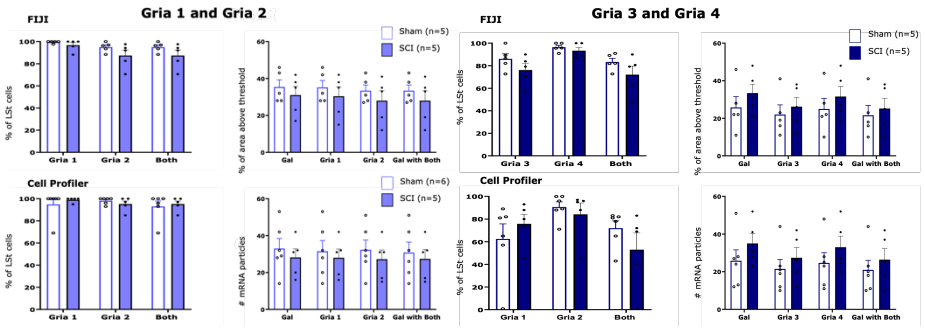
Subtask 3.2 (Aim 2B): To test if SCI-impaired ejaculatory reflex is restored by glutamate infusions.

Final Progress: Studies were completed to test that LSt cells express AMPA receptors and that AMPA receptor function is essential to trigger ejaculatory reflexes in sham control animals. Similar to Subtask 3.1, during the final reporting period, findings from the previous budget year were replicated in separate cohorts of subjects and data were re-analyzed and presented for final presentation of data. The findings below are idescribed in a manuscript currently in final stage of preparation for submission.

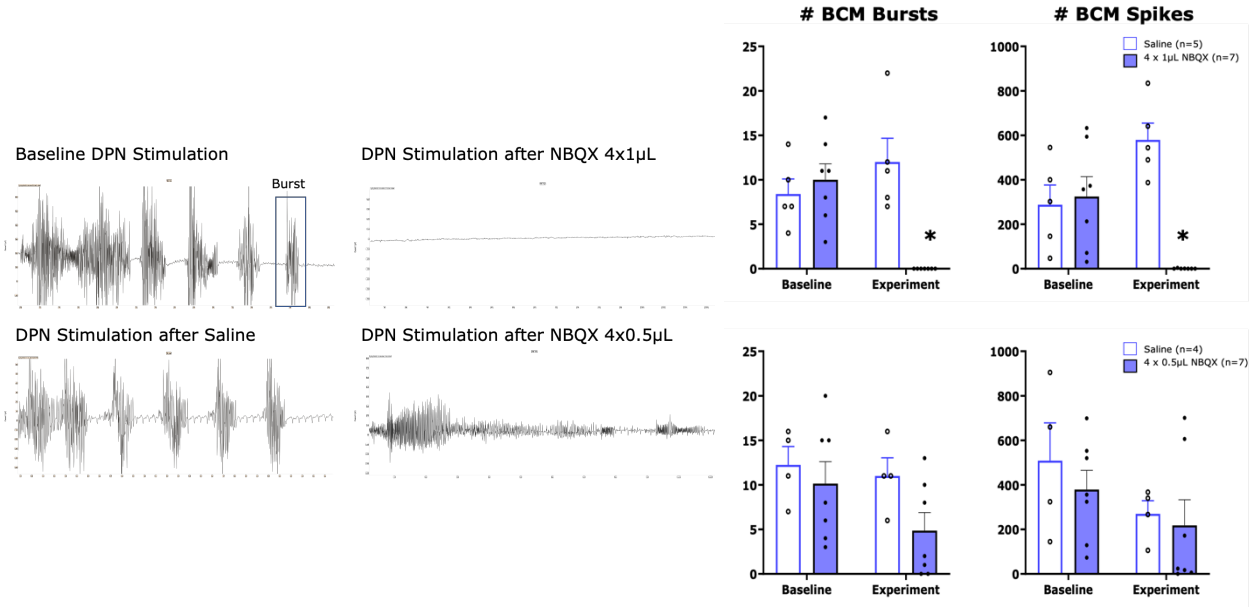
1. Analysis of AMPA receptor mRNA expression in the lumbosacral spinal cord using fluorescent In Situ Hybridization using RNAscope showed that

- LSt cells express all four GluA receptor subunits in Sham animals.
- SCI had no effects on percentages of LSt cells expressing GluA receptor subunits.
- SCI had no effect on GluA receptor subunit expression within LSt cells, based on fluorescent signal densitometry (Fiji), or analysis of numbers of mRNA particles (Cell Profiler).





2. Intraspinal infusions of AMPA antagonist NBQX into the area of LSt cells prevented ejaculatory reflexes upon stimulation of the sensory nerve (Dorsal penile nerve) in all animals.



In conclusion:

- These data demonstrate that the majority of LSt cells co-express several GluA receptor subunits.
- The activation of AMPA receptors is essential for the ejaculation-triggering effects of sensory nerve stimulation.
- In combination with past data, these results indicate that both NMDA and AMPA receptors are necessary for sensory processing in LSt cells required for ejaculation.
- A caveat of the current study is that intraspinal injections of NBQX likely targeted neurons other than LSt cells. Thus, effects of AMPA receptor blockade may be independent of actions specifically in LSt cells. Future studies using cell-specific manipulations will be needed.

Despite these interesting results pertaining basic function of the SEG in sham control animals, we were unable to translate these findings to pharmacological manipulations in SCI rats, due to the abundance of different AMPA receptors in cells surrounding the LSt cells and the lack of specific receptor targeting agonist, creating a high likelihood of side effects and neuronal toxicity with agonist treatment. Thus, the question if GluA receptor subunits in SCI rats using specific receptor modulators can restore ejaculatory function remains untested.

Opportunities for training and professional development

Training and development were offered to PhD student (T. Ettey) and Postdoctoral scholar (E. Brown), including attendance of National Neurotrauma Symposium and Society for Neuroscience conferences, local annual symposium of the Brain Health Research Institute at Kent State University, and numerous webinars and training modules offered virtually. Moreover, several undergraduate students benefited from these studies as they were mentored by PhD student (T. Ettey) and Postdoctoral scholar (E. Brown) during summer undergraduate research experiences.

Dissemination of results:

Dr. Coolen has delivered several oral presentations including at the Neurotrauma Symposium of the National Neurotrauma Society in June 2023 and has presented findings of the studies included in this project. PhD student (T. Ettey) and Postdoctoral scholar (E. Brown) presented numerous poster presentations at National Neurotrauma Symposium and Society for Neuroscience conferences, and local annual symposium of the Brain Health Research Institute at Kent State University.

4. Impact

Thus far we have provided evidence in support of the hypothesis that chronic spinal cord injury causes long term deficits in transmission of sensory inputs related to sexual activity to the spinal ejaculation generator. These findings have an impact on our knowledge of the long-term effects of spinal cord injury in general, and on the impact that spinal cord injury has on ejaculatory dysfunction in particular.

5. Changes/Problems

Nothing to report.

6. Products

Two manuscripts are currently in final stage of reparation but haven't yet been submitted.
Abstracts that were submitted or presented during the final reporting year:

- Thywill Ettey, Nathan Ritchey, Lique Coolen. Spinal cord GluA receptor activation is required for ejaculatory reflexes in adult male rats. Neuroscience 2022, National Neurotrauma Society annual meeting, Austin, TX. June 25-28, 2023. Abstract# 453. Poster # 125.05
- Eric V. Brown, Nathan P. Ritchey, Nathan J. Mudrak, Thywill H. Ettey, and Lique M Coolen Sexual dysfunction following chronic spinal cord injury is associated with glutamatergic deficits in the spinal ejaculation generator National Neurotrauma Society annual meeting, Austin, TX. June 25-28, 2023. Abstract# 451. Poster # P02.38
- Thywill Ettey, Nathan Ritchey, Lique Coolen. Spinal cord GluA receptor activation is required for ejaculatory reflexes in adult male rats. Society for neuroscience annual meeting, San Diego, CA. November 12-16, 2022. Abstract # 453. Poster # P02.39.
- Eric V. Brown, Nathan P. Ritchey, Nathan J. Mudrak, Thywill Ettey, Kiran K. Koni, and Lique M. Coolen. Contusion spinal cord injury decreases glutamatergic axon inputs to spinal ejaculation generator in male rats. Neuroscience 2022, Society for neuroscience annual meeting, San Diego, CA. November 12-16, 2022.
- Thywill Ettey, Nathan Ritchey, Lique Coolen. Spinal cord GluA receptor activation is required for ejaculatory reflexes in adult male rats. BHRI 10th Annual Neuroscience Symposium at Kent State University. October 27-29, 2022. Abstract and Poster # 17
- Eric V. Brown, Nathan P. Ritchey, Nathan J. Mudrak, Thywill Ettey, Kiran K. Koni, and Lique M. Coolen. Contusion spinal cord injury decreases glutamatergic axon inputs to spinal ejaculation generator in male rats. BHRI 10th Annual Neuroscience Symposium at Kent State University. October 27-29, 2022. Poster #43.

7. Participants & Other Collaborating Organizations

Name:	*Lique M Coolen*
Project Role:	*Project Director*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID 0000-0003-2920-1116*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Coolen oversaw all aspects of the projects and has supervised and trained the Research Technician and graduate student.*
Funding Support:	*N/A*

Name:	*Eric Brown*
Project Role:	*Postdoctoral Scholar*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Brown was involved in all aspects of these studies*
Funding Support:	*N/A*

Name:	*Thywill Ettey*
Project Role:	*PhD Student- Graduate Assistant-Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Ms. Ettey conducted pharmacological studies and was involved in all aspects of these studies.*
Funding Support:	*N/A*

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

No changes; Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. Special Reporting Requirements

Nothing to Report.

9. Appendices

None included.