AWARD NUMBER: W81XWH-10-1-0715

TITLE: Magnetic Resonance Characterization of Axonal Response to Spinal Cord Injury

PRINCIPAL INVESTIGATOR: Alan Tessler, M.D.

CONTRACTING ORGANIZATION: Drexel University College of Medicine
Philadelphia, PA 19104

REPORT DATE: February 2014

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED *(From - To)*
February 2014	FINAL	27Sep2010 - 26Sep2013

3. TITLE AND SUBTITLE

Magnetic Resonance Characterization of Axonal Response to Spinal Cord Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0715

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Alan Tessler, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Drexel University
Office of Research Compliance and Admin.
3201 Arch Street
Philadelphia, PA 19104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The animal model will be implemented at DUCOM. The investigators at DUCOM, Drs. Tessler, Himes and Murray, have extensive experience in spinal cord injury. They will supervise the creation of the cervical spine injuries, care of the animals, sacrifice and perfusion fixation, and, after completion of imaging, histologic preparation of the specimens.

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	UU	5	19b. TELEPHONE NUMBER *(include area code)*

Taken from the *Statement of Work* in the original submission:

Drexel University College of Medicine
The animal model will be implemented at DUCOM. The investigators at DUCOM, Drs. Tessler, Himes and Murray, have extensive experience in spinal cord injury. They will supervise the creation of the cervical spine injuries, care of the animals, sacrifice and perfusion fixation, and, after completion of imaging, histologic preparation of the specimens.

All parts of the above statement of work were completed as detailed below with the exception of the histologic preparation of the specimens (which was designed to take place after completion of the MRI imaging). There was an extensive delay in installation and validation of the new MRI hardware and software at U Penn. Imaging of spinal cord specimens sent to U Penn was significantly delayed. Spinal cords were stored in fixative for many months to years, rendering the specimens unusable for many of the proposed histology procedures. Alternate plans were discussed but none of the spinal cords were returned to DUCOM for histologic preparation.

U Penn and DUCOM personnel met in November, 2010 to discuss the appropriate type of spinal cord injury (SCI) in experimental mice, time courses for survival of the SCI mice and preparation of spinal cord tissue for MRI and subsequently for comparison with histological analysis. In particular, optimal choice of tissue fixative was discussed to match both imaging and histology. The lab standard of 4% paraformaldehyde gives reasonable structural integrity for MRI but does not overly fix the tissue. This was important so that the proposed histological analysis could be performed on sectioned tissue after MRI. In addition, 2% paraformaldehyde with 2.5% glutaraldehyde, a stronger fixative that results in good MRI images but compromises the histological staining in some cases, was also considered. This stronger fixative has been used successfully in previous studies from our labs (see Schwartz, et.al. Ex vivo evaluation of ADC values within spinal cord white matter tracts. AJNR Am J Neuroradiol. 2005 Feb;26 (2):390-7).

The first four mice were prepared and sacrificed in November, 2010. These animals had no spinal cord injury (SCI) and were prepared as controls to test the 2 types of fixative. Spinal cords were collected and preserved in fixative until the Penn MRI team was ready for the tissue. An MRI unit at U Penn was functional in early February, 2011 and control cords were imaged to refine technical details of the procedure.

The next group of 4 mice received a partial spinal hemisection in January, 2011. This lesion at spinal level C4 spared the dorsal funiculus (corticospinal tract) and ventral spinal cord while targeting specific spinal pathways that could be imaged for changes in axonal density and myelin content in white matter after SCI. Sacrifice for this group was planned for 3 weeks post-injury but, because the MRI unit at Penn was scheduled for upgrades (4 – 6 week delay), sacrifice of these mice was delayed until early April, 2011. All surgeries were performed by experienced investigators under approved Drexel University IACUC protocol #19180 and pre-and post-op animal care was provided by members of our staff experienced in the care of mice. All surgeries were performed under an anesthetic cocktail of Ketamine and Xylazine at a dose of 0.0012cc/g. Mice with a C4 partial injury show minimal functional deficits and do not require extensive post-operative care beyond analgesia, fluids and antibiotics in the acute post-op period, daily observation in the sub-acute post-op period and weekly observation in the chronic post-op period. The animals were observed eating, drinking and moving around the cage within 3-4 hours of surgery. Mice were house 2-3 per cage for the duration of the

experiment. Upon sacrifice, half of the animals were perfused with the standard fixative described above and half with the stronger fixative.

Additional 2 mice received the C4 partial spinal hemisection lesion and were sacrificed in February, 2011 for analysis at the acute, 2 day time point post-injury.

A second meeting of U Penn and DUCOM personnel occurred in February, 2011. Ongoing issues about lesion location, area(s) to be imaged, how to specifically mark the cord to show imaged areas, optimal time points for analysis and a new plan for histological techniques that could be used to measure changes in myelin thickness after SCI were discussed. However, optimal immunohistochemical staining could not be achieved in tissue that soaked in fixative for months, as was the case for the animals that were previously prepared and waiting for imaging. It was decided to keep the lesion at the C4 spinal level and to eliminate the 2-day time point from analysis because MRI imaging in the very acute post-op period would be too difficult to interpret. It was also decided to continue using both types of fixatives to maximize options for histological analyses post- MRI.

Another group of 6 mice received the partial spinal hemisection injury in early February, 2011 and were sacrificed in April, 2011.

The final group of 6 mice received a partial C4 spinal hemisection lesion in mid-April, 2011 and were sacrificed in early May, 2011 for analysis at the sub-acute of 3-week time point.
Spinal cords of 2-day, 3-week and 3-month post-injury animals (10 total) were delivered to U Penn for MRI analysis.

Hardware modifications for the final installation of the new Brucker system at U Penn and delays in development of a QSI pulse sequence program (their old program couldn't run on the new system) persisted into the summer of 2011. We contacted a former DUCOM colleague, Dr. Yoni Nissanov, who developed a program for axon segmentation analysis used in previous work with U Penn (see Chin CL, Wehrli FW, Fan Y, Hwang SN, Schwartz ED, Nissanov J, Hackney DB. Assessment of axonal fiber tract architecture in excised rat spinal cord by localized NMR q-space imaging: simulations and experimental studies. Magn Reson Med. 2004 Oct;52(4):733-40). Unfortunately, the software application was proprietary and Dr. Nissanov required a 15K/year subcontract to move forward with its use.

The remaining 12 spinal cords (control, 3 week and 2-3 month survival times) that had been stored in fixative for 6 months to 1 year were delivered to U Penn for MRI analysis in February, 2012. Further delays with installation of the new system at U Penn stalled the analysis of new material and personnel changes set them back even further.
In March of 2014, we were informed that that a web-based interface interface and processing program was in development at U Penn and that the sectioning and staining of the spinal cord that had undergone MRI analysis would be done in a histology core lab at U Penn.

In August of 2014, we were given one slide prepared by the U Penn histology core lab of 1µm cross sections from a control spinal cord stained with toluidine blue. The cord was photographed at DUCOM and images were sent to U Penn for quantitative image analysis. The file with these images can be viewed at:

https://urldefense.proofpoint.com/v1/url?u=https://www.dropbox.com/l/XurAf689JCqaEgxf4sLQ9v&k=mz4A1tcEqbGYGaLi8qkyuQ%3D%3D%0A&r=8nT2aYpHejiE9zKyuMMpTu%2FCHKeFqh6eYfVlBofPgZw%3D%0A&m=OQA8Hgn6MDoy7o0pXOP%2FxWo3w6bFO8c9Z69FI2vPmKI%3D%0A&s=98fe20f0f7d94513f777165a19650bc38206d62b40b72112cf8165341896d898>.

We had originally hired a 50% technician to section and stain the spinal cords but she had left Drexel by this time. We asked Dr. Wehrli if he could provide funds to hire another person, as we were not satisfied with the preservation of the specimen that he showed us. Dr. Wehrli said he would ask the facility at Penn to prepare and analyze the tissue. No other samples or images were shared with us.

Technician Michelle Klaw and co-PI Marion Murray received pay from the research effort. No publication resulted from this project.