

AWARD NUMBER: W81XWH-16-1-0605

TITLE: **MRI Diffusion Tensor Tractography to Track and Monitor
Peripheral Nerve Recovery after Severe Crush or Cut/Repair Nerve
Injury**

PRINCIPAL INVESTIGATOR: Wesley Thayer, MD/PhD (Vanderbilt)

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center, D4207 MCN, Dept. of
Plastic Surgery, VUMC, Nashville, TN 37232

REPORT DATE: April 2021

TYPE OF REPORT: Final Technical Report

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE APRIL 2021		2. REPORT TYPE Final		3. DATES COVERED 1 Sep 2016 - 31 DEC 2020	
4. TITLE AND SUBTITLE MRI Diffusion Tensor Tractography to Track and Monitor Peripheral Nerve Recovery after Severe Crush or Cut/Repair Nerve Injury				5a. CONTRACT NUMBER W81XWH-16-1-0605	
				5b. GRANT NUMBER MR150075	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Wesley P. Thayer MD/PhD E-Mail: wesley.thayer@vumc.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Vanderbilt University Medical Center, D4207 MCN, Dept. of Plastic Surgery, VUMC, Nashville, TN 37232				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 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 Nerve regeneration after an injury should occur in a timely fashion for function to be restored. Failed repairs can take up to a year to detect for proximal injuries precluding successful re-operation. Methods: Sprague-Dawley rats were utilized to assess recovery after nerve injury and MRI analysis was compared to behavioral and histologic measures. Using preclinical studies to guide imaging, human subjects were recruited and scanned following TPNI and subsequent repair. Results: DTI can non-invasively differentiate crush and cut injuries, and normalized indices do so as early as 4 weeks. Furthermore, high resolution DTI may provide viable biomarkers of recovery as early as 1 week. DTI was also able to distinguish TPNI from more minor trauma. The use of DTI ellipsoids analysis can be used to distinguish injury severity. Conclusions: DTI application to TPNI provides a non-invasive method of determining if patient recovery is progressing as expected or if revisional surgery is required. This could lead to earlier intervention and improved outcomes for patients with devastating nerve injuries. In fact, in our human dataset, a lack of improvement was noted in one patient, followed by rapid recovery post revision surgery. This supported the findings made in the MRI analysis.					
15. SUBJECT TERMS Traumatic Peripheral Nerve Injury; TPNI; Axonal Repair; Nerve Regeneration; MRI; Diffusion Tensor Imaging; DTI; quantitative MRI					
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	Unclassified	57	19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

Page

1. Introduction
2. Keywords
3. Accomplishments
4. Impact
5. Changes/Problems
6. Products
7. Participants & Other Collaborating Organizations
8. Special Reporting Requirements
9. Appendices

1. INTRODUCTION:

Nerve injuries to the upper and lower extremities are very common in the United States in trauma patients, occurring in over 17,500 people annually. In the military, there is a greater than 90% chance of survival from a battlefield injury. Unfortunately, the increasing rate of survival means that more wounded warriors are living the rest of their lives with injured arms and legs or amputations. Many of these injuries involve damage to nerve(s) with remarkable loss of function caused due to denervation. In severe cases, despite nerve repair, these injuries often heal poorly, resulting in extremities with limited function. This study seeks to develop a novel MRI-based strategy to monitor nerve recovery after injury and surgical repair to intervene in situations where nerves are not recovering appropriately. MRI is non-invasive, while current methods of following nerve injury, such as EMG and nerve conduction studies, are invasive and only offer limited information about the repairs. This project is unique in that it proposes a synergistic collaboration between i) a peripheral neurosurgeon with expertise in nerve repair and ii) engineers with expertise in developing and translating advanced MRI approaches for peripheral nerve applications.

2. KEYWORDS: Traumatic Peripheral Nerve Injury; TPNI; Axonal Repair; Nerve Regeneration; MRI; Diffusion Tensor Imaging; DTI; quantitative MRI

3. ACCOMPLISHMENTS:

▪ What were the major goals of the project?

The overall objective of these studies is to evaluate the ability of DTI to monitor and, more importantly, predict nerve regrowth following crush or cut with surgical repair. Animal studies will be performed with DTI results compared to measures of axonal recovery from electrophysiology and histology; and validated protocols derived from these animal studies will be translated into humans. We hypothesize that the additional information available via DTI will improve our ability to monitor and predict nerve regrowth following surgical repair or severe crush injury, guiding clinical management either toward or away from surgical intervention.

Aim 1. Determine if DTI fiber tracking can predict recovery of peripheral nerve function after crush or cut/repair peripheral nerve injury with repair in a rat sciatic nerve model. Graded injuries to either sensory, motor, or mixed nerves will be separately evaluated. Nerve conduits and grafts will be compared to standard repair.

Task 1: DTI fiber tracking after crush or cut/repair peripheral nerve injury: rat sciatic model

Task 2: Graded mixed nerve injury recovery will also be evaluated: rat sciatic nerve model

Task 3: Nerve conduits and grafts will be compared to standard repair: rat sciatic nerve model

Aim 2. Determine if our DTI fiber tracking protocol can predict recovery of peripheral nerve function in human case studies (crush or cut with repair). Pure sensory, motor, and mixed nerve injuries will be evaluated.

Task 1: Recruit control subjects

Task 2: Recruit nerve injury cases

<u>Specific Aim 1</u> (DTI imaging of nerve injuries in our animal model)	Timeline	Thayer Lab	Dortch/Does Lab
Task 1: DTI after cut/crush injury	Months		
Nerve injury, repair, and histologic, behavioral, and MRI analysis of severed nerves.	6-9	+	+
Milestone(s) Achieved		<i>Animals completed 04-19-2017. Analysis continued into late 2018</i>	
Local IACUC Approval	3	+	+
Milestone Achieved: HRPO/ACURO Approval	6	<i>Local IACUC received 06-2-2016 ACURO received 06-8-2016</i>	
Task 2: Graded Injury			

Specific Aim 1 (DTI imaging of nerve injuries in our animal model)	Timeline	Thayer Lab	Dortch/Does Lab
Nerve injury, repair, and histologic, behavioral, and MRI analysis of nerves severed nerves with 25%, 50%, or 75% transection.	9-12	+	+
Milestone(s) Achieved:		<i>Animals completed 08-17-2018. Analysis continued into late 2020.</i>	
Task 3: Conduit and nerve graft repairs			
Conduits and grafts compared to standard repair	9-12	+	+
Milestone(s) Achieved:	12	Incomplete.	
Final Data Analysis/Publication:	30-36	+	+
Milestone(s) Achieved		Three manuscripts published: 2019 Dec 23;9(1):19686. doi: 10.1038/s41598-019-56215-2 2020 Apr;145(4):949-956. doi: 10.1097/PRS.00000000000006638 2020 Jan 7;185(Suppl 1):35-41. doi: 10.1093/milmed/usz360	
Specific Aim 2 DTI fiber tracking in human case studies (crush or cut with repair).	Months		
Task 1: Recruit control subjects			
Normal subjects will be recruited to undergo MRI and EMG/NCS evaluation	6-9	+(24 total)	+
Milestone(s) Achieved		<i>Enrollment began 01-06-2017</i>	
Local IACUC Approval	3	+	+
Milestone Achieved: HRPO/ACURO Approval	6	<i>Local IRB received 07-14-2016 HRPO received 10-20-2016</i>	
Task 2: Recruit nerve injury cases			
Recruit 24 mixed nerve repair cases	12-24	+	+
Recruit 10 sensory or motor nerve repair cases	12-30	+	+
Milestone(s) Achieved		<i>Enrollment began 01-06-2017</i>	
Final Data Analysis/Publication:	30-36	+	+
Milestone(s) Achieved		Manuscript published 2021 February; 8(2):332-347. doi: 10.1002/acn3.51270	

Incompatible processing requirements for different outcome measures led to increased number of animals being required for Task 1 and 2. Delays in development and manufacturing of a reproducible device for consistently reproducing the desired graded injuries took more time than initially anticipated. As a result of reviewer comments and suggestions while undergoing the manuscript publication process, analysis of Task 1 animals continued through late 2018. The same has been true for Task 2, as analysis has continued into early 2021.

▪ **What was accomplished under these goals?**

- All animal experiments for task 1 and 2 were successfully completed.
- Three manuscript concerning the animal work have been published to date and a fourth is currently undergoing revisions for publication.
- Data generated from Aim 1, Task 1 showed that DTI can be used to differentiate crush and cut injuries. Further development of this finding could provide a non-invasive method of determining if patient suffering from TPNi and repair are progressing as expected or if revisional surgery is required, as approximately 40% of all repairs fail.

Farinas AF, Manzanera Esteve IV, Pollins AC, Cardwell NL, Kaoutzanis C, Nussenbaum ME, Does MD, Dortch RD, Perdakis G, Thayer WP. Diffusion Magnetic Resonance Imaging Predicts Peripheral Nerve Recovery in a Rat Sciatic Nerve Injury Model. *Plast Reconstr Surg.* 2020 Apr;145(4):949-956. doi: 10.1097/PRS.0000000000006638. PubMed PMID: 32221212; PubMed Central PMCID: PMC7270143.

- Data generated from Aim 1, Task 1 also showed that normalized indices from high-resolution *ex vivo* DTI methods of MRI analysis could differentiate crush from cut/repair injuries as early as 4 weeks once edema has receded. More complex higher order DKI models, which take longer, did not provide any additional benefit. Further study in *in vivo* models would help to prove that these indices can serve as biomarkers of recovery in TPNI patients.

Manzanera Esteve IV, Farinas AF, Pollins AC, Nussenbaum ME, Cardwell NL, Kang H, Does MD, Thayer WP, and Dortch RD. Probabilistic Assessment of Nerve Regeneration with Diffusion MRI in Rat Models of Peripheral Nerve Trauma. *Sci Rep.* 2019 Dec 23;9(1):19686. doi: 10.1038/s41598-019-56215-2. PubMed PMID: 31873165; PubMed Central PMCID: PMC6928159.

- Data generated from Aim 1, Task 2 showed that orientation information from high resolution DTT may provide viable biomarkers of TPNI recovery as early as 1 week, despite the presence of edema. Though scalar indices are still corrupted at this early time point, the use of DTI ellipsoids analysis can be used to distinguish injury severity.

Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Nuessenbaum ME, Does MD, Dortch RD, Thayer WP. Diffusion Tensor Tractography Visualizes Partial Nerve Laceration Severity as Early as 1 Week After Surgical Repair in a Rat Model *Ex Vivo*. *Mil Med.* 2020 Jan 7;185(Suppl 1):35-41. doi: 10.1093/milmed/usz360. PubMed PMID: 32074298; PubMed Central PMCID: PMC7029835.

- Data generated from Aim 1, Task 2 is still undergoing the publication process. We learned that partial cut injuries healed via 2 different methods which each presented in slightly different ways. Minor injuries resulted in healing via robust collateral sprouting while severe injuries healed primarily from distal sprouting. This is supported in Figure 1 below where axon density in the injured regions of 25% transect nerves is increased over uninjured regions.

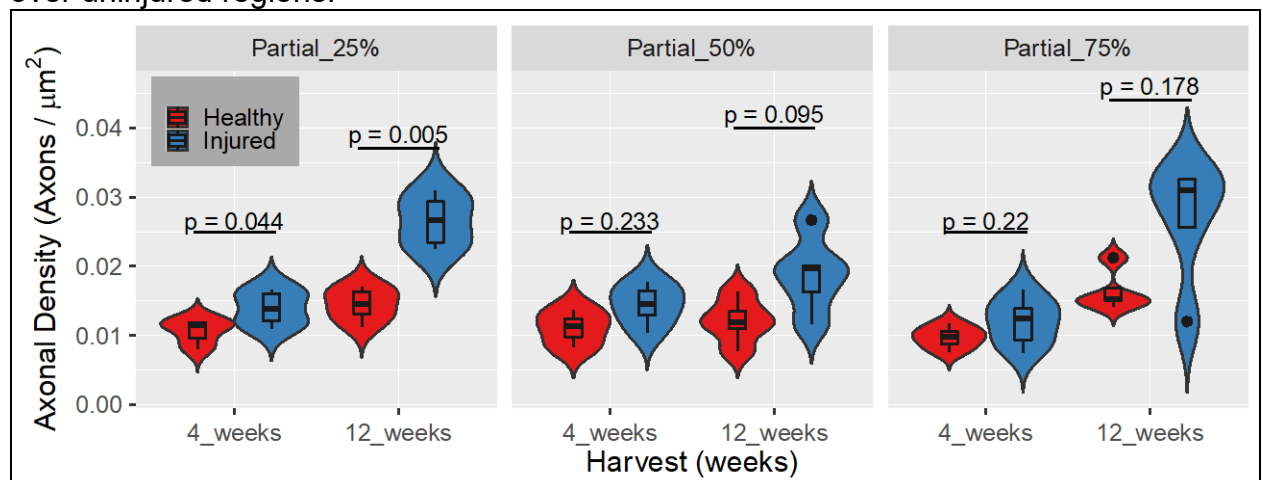


Figure 1: Violin plots of axon density in areas of healthy (uninjured) vs injured regions distal to the injury/repair site of partially transected nerves.

- The 75% injured nerves healed primarily via distal sprouting which is not as robust as collateral sprouting. However, the 50% transected group seemed to be the worst based on total axon counts alone. This non-linear pattern was an unexpected finding. When we looked closer at the axon densities, we discovered for the 50% injured group that there was broader variability and that the density of the uninjured regions was impacted as well. Therefore at 12 weeks you can see that densities of the 25% and 75% are roughly the same, 50% is a bit behind. This supports the clinical observation that overall recovery time can be lessened through surgical resection when a moderate injury is discovered.
- We learned that radial diffusivity (RD), one of the DTI scalar indexes has very good correlation with axonal density (Figure 2). This is a promising biomarker which could be used as a non-invasive method to determine if a traumatic injury has resulted in nerve severance severe enough to require surgical intervention.

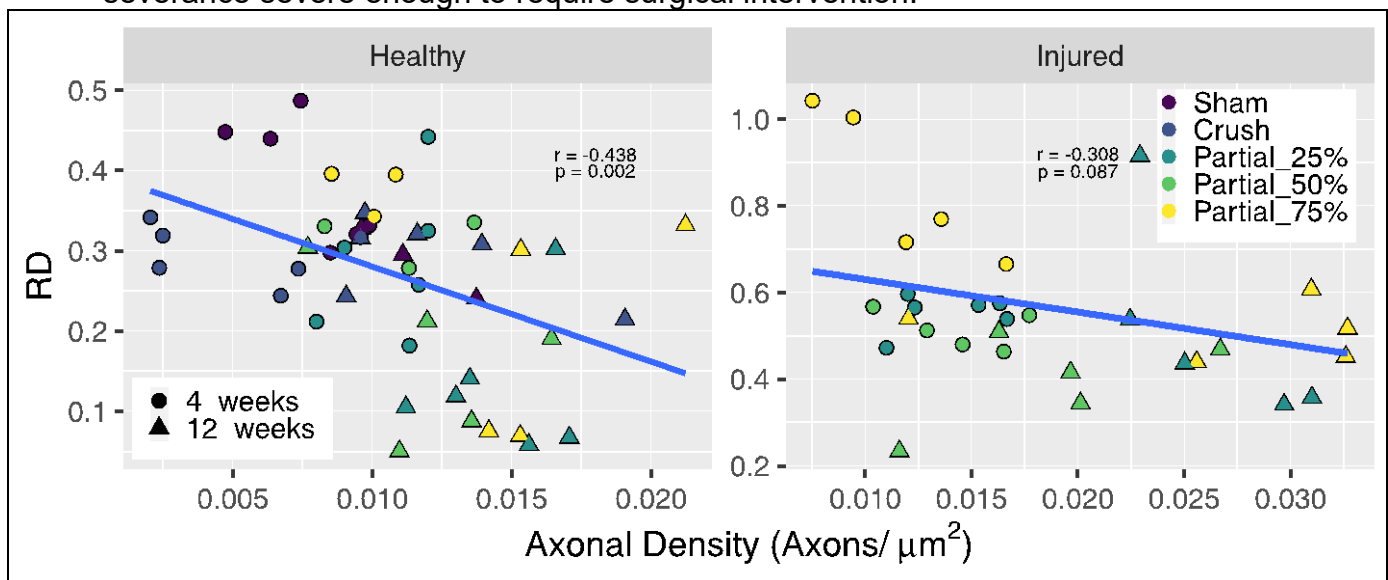


Figure 2: Correlations between RD and every group at both timepoints.

- Data generated from Aim 2, showed that DTI could distinguish TPNI from more minor trauma, such as carpal tunnel syndrome. Furthermore, a lack of improvement was displayed in one patient, followed by rapid recovery post revision surgery. This was one powerful display of the usefulness of this technology in the clinical setting. Additionally, we showed that similar to what we observed in the *ex vivo* animal experimentation, fractional anisotropy (FA) while valuable is mostly driven by RD even in the *in vivo* clinical setting.

Pridmore M, Glassman G, Pollins AC, Manzanera Esteve IV, Drolet BC, Weikert D, Does MD, Perdakis G, Thayer WP, Dortch RD. Initial Findings in Traumatic Peripheral Nerve Injury and Repair with Diffusion Tensor Imaging. *Annals of Clinical and Translational Neurology*. 2021 February; 8(2):332-347. doi: 10.1002/acn3.51270. Online ahead of print 2021 Jan 6. PMID: 33403827

- **What opportunities for training and professional development has the project provided?**
This grant helped advance the training directly of 2 research fellows, both of whom are now in plastic surgery training and supported publication and research opportunities for Dr Manzanera who was able to join VUMC as junior faculty as a result of the success of this grant. Moreover, Dr Thayer has been promoted to Vice Chair of Research for the Department of plastic Surgery,

and Dr Dortch has been able to take a promotion at Barrow Institute as Associate Professor in Phoenix, AZ.

▪ **How were the results disseminated to communities of interest?**

1. Manzanera IV, Farinas AF, Pollins AC, Cardwell NL, Nussenbaum ME, Does MD, Thayer WP, Dortch RD. Diffusion Tensor Imaging of Excised Rat Nerve Following Transection and Surgical Repair. Vanderbilt University Institute of Imaging Sciences Annual Retreat, June 2018, Nashville, TN (Poster)
2. Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Nuessenbaum ME, Does MD, Dortch RD, Thayer WP. MRI DTI Tractography Can Identify Early Partial Injuries in a Rat Sciatic Nerve Model. Military Health Systems Research Symposium, August 20-23, 2018, Kissimmee, FL (Podium)
3. Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Dortch RD, Thayer WP. Developing a Fraction Anisotropy Cutoff Value to Diagnose Recovery in Peripheral Nerve Injury. ASPN Annual Meeting, February 1-3, 2019, Palm Desert, CA. (Podium)
4. Manzanera Esteve IV, Farinas AF, Pollins AC, Nussenbaum ME, Cardwell NL, Kang H, Does MD, Thayer WP, and Dortch RD. Probabilistic Assessment of Nerve Regeneration with Diffusion MRI in Rat Models of Peripheral Nerve Trauma. Sci Rep. 2019 Dec 23;9(1):19686. doi: 10.1038/s41598-019-56215-2. PubMed PMID: 31873165; PubMed Central PMCID: PMC6928159.
5. Farinas A, Manzanera IE, Pollins A, Glassman G, Dortch RD, Thayer WP. Diffusion Tensor Imaging as a diagnostic tool to assess partial nerve laceration severity and recovery in a rat model ex vivo. ASPN Annual Meeting, January 10-12, 2020, Ft. Lauderdale, FL (ePoster).
6. Farinas AF, Manzanera Esteve IV, Pollins AC, Cardwell NL, Kaoutzanis C, Nussenbaum ME, Does MD, Dortch RD, Perdakis G, Thayer WP. Diffusion Magnetic Resonance Imaging Predicts Peripheral Nerve Recovery in a Rat Sciatic Nerve Injury Model. Plast Reconstr Surg. 2020 Apr;145(4):949-956. doi: 10.1097/PRS.0000000000006638. PubMed PMID: 32221212; PubMed Central PMCID: PMC7270143.
7. Manzanera IE, Farinas A, Pollins A, Thayer WP, Dortch RD. Assessment with Partial Peripheral Nerve Transection with Diffusion MRI. ISMRM Annual Meeting, April 17-20, 2020, Sydney, NSW, Australia (Poster), Meeting cancelled due to COVID-19.
8. Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Nuessenbaum ME, Does MD, Dortch RD, Thayer WP. Diffusion Tensor Tractography Visualizes Partial Nerve Laceration Severity as Early as 1 Week After Surgical Repair in a Rat Model Ex Vivo. Mil Med. 2020 Jan 7;185(Suppl 1):35-41. doi: 10.1093/milmed/usz360. PubMed PMID: 32074298; PubMed Central PMCID: PMC7029835.
9. Pridmore M, Glassman G, Pollins AC, Manzanera Esteve IV, Drolet BC, Weikert D, Does MD, Perdakis G, Thayer WP, Dortch RD. Initial Findings in Traumatic Peripheral Nerve Injury and Repair with Diffusion Tensor Imaging. Annals of Clinical and Translational Neurology. 2021 February; 8(2):332-347. doi: 10.1002/acn3.51270. Online ahead of print 2021 Jan 6. PMID: 33403827

▪ **What do you plan to do during the next reporting period to accomplish the goals?**

N/A the grant is complete however we still have a publication under review.

4. IMPACT:

▪ **What was the impact on the development of the principal discipline(s) of the project?**

The data and work generated from this award has increased our knowledge and therefore the scientific community's knowledge on the usefulness of DTI within the field of TPNI repair and recovery. The rat studies proved that a single scan analyzed via a variety of methods can be used to distinguish multiple gradients of injury from one another and can be utilized as a tool to grade healing rates. The *in vivo* human observations of the clinical trial support what we have discovered in the animal models. The next logical step of this research is to expand DTI imaging after TPNI to a multi-center clinical trial based on the success of this work. would not be possible without the advancements made through this funded work.

- **What was the impact on other disciplines?**

As the research evolved, so too did our DTI analysis of the data. As we applied the traditional DTI techniques to our experimental data, we found that we needed to modify the methods to better fit the model. As we learned more about the environment of the peripheral nerve, we adapted tools typically used for MRI analysis of other tissues to better fit our model and then we validated those techniques. We are confident that the methods developed from this work have advanced the peripheral nerve research discipline and have broad applicability within that field.

- **What was the impact on technology transfer?**

The data from this work has the potential to change TPNI clinical practice in the future. As a non-invasive method for detecting recovery after surgical intervention, MRI/DTI has the potential to identify failed repairs earlier in the recovery process than traditional methods, which will then allow critical revision surgery increasing the likelihood of successful treatment and reducing the emotional and physical pain associated with unsuccessful treatment. Further study will be needed to verify these findings.

- **What was the impact on society beyond science and technology?**

Nothing to report.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Human recruitment was slower than anticipated. We aggressively sought out a clinical research team member to facilitate patient recruitment. We succeeded in finding and training a suitable candidate, but unfortunately, they later had to leave for personal reasons. Later in the study, the COVID-19 pandemic forced safety measures to be implemented and we were unable to get our patients back into the research facility to be scanned.

Our animal experimentation fortunately produced ample amounts of useful data and though we would have preferred to have even more human data to focus on, we were able to dedicate our time to publishing plethora of information from our successful animal experiments and our clinical studies. The pandemic created many challenges, but we were able to continue our analysis efforts from remotely, while staying safe.

- **Changes that had a significant impact on expenditures**

Nothing to report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

- **Significant changes in use or care of human subjects**

Nothing to report.

- **Significant changes in use or care of vertebrate animals**

Nothing to report.

- **Significant changes in use of biohazards and/or select agents**

Not applicable

6. PRODUCTS:

▪ Publications, conference papers, and presentations

- **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*
 - Manzanera Esteve IV, Farinas AF, Pollins AC, Nussenbaum ME, Cardwell NL, Kang H, Does MD, Thayer WP, and Dortch RD. Probabilistic Assessment of Nerve Regeneration with Diffusion MRI in Rat Models of Peripheral Nerve Trauma. *Sci Rep.* 2019 Dec 23;9(1):19686. doi: 10.1038/s41598-019-56215-2. PubMed PMID: 31873165; PubMed Central PMCID: PMC6928159. Published with acknowledgement of federal support.
 - Farinas AF, Manzanera Esteve IV, Pollins AC, Cardwell NL, Kaoutzanis C, Nussenbaum ME, Does MD, Dortch RD, Perdakis G, Thayer WP. Diffusion Magnetic Resonance Imaging Predicts Peripheral Nerve Recovery in a Rat Sciatic Nerve Injury Model. *Plast Reconstr Surg.* 2020 Apr;145(4):949-956. doi: 10.1097/PRS.0000000000006638. PubMed PMID: 32221212; PubMed Central PMCID: PMC7270143. Published with acknowledgement of federal support.
 - Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Nuessenbaum ME, Does MD, Dortch RD, Thayer WP. Diffusion Tensor Tractography Visualizes Partial Nerve Laceration Severity as Early as 1 Week After Surgical Repair in a Rat Model Ex Vivo. *Mil Med.* 2020 Jan 7;185(Suppl 1):35-41. doi: 10.1093/milmed/usz360. PubMed PMID: 32074298; PubMed Central PMCID: PMC7029835. Published with acknowledgement of federal support.
 - Pridmore M, Glassman G, Pollins AC, Manzanera Esteve IV, Drolet BC, Weikert D, Does MD, Perdakis G, Thayer WP, Dortch RD. Initial Findings in Traumatic Peripheral Nerve Injury and Repair with Diffusion Tensor Imaging. *Annals of Clinical and Translational Neurology.* 2021 February; 8(2):332-347. doi: 10.1002/acn3.51270. Online ahead of print 2021 Jan 6. PMID: 33403827. Published with acknowledgement of federal support.
 - Manzanera Esteve IV, Farinas AF, Pollins AC, Nuessenbaum ME, Cardwell NL, Kang H, Does MD, Dortch RD*, Thayer WP*. Noninvasive Diffusion MRI to determine the severity of peripheral nerve injury. *Magnetic Resonance Imaging Journal.* Under review with acknowledgement of federal support.
- **Books or other non-periodical, one-time publications.**
Nothing to report.
- **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.*
 - Manzanera IV, Farinas AF, Pollins AC, Cardwell NL, Nussenbaum ME, Does MD, Thayer WP, Dortch RD. Diffusion Tensor Imaging of Excised Rat Nerve Following Transection and Surgical Repair. Vanderbilt University Institute of Imaging Sciences Annual Retreat, June 2018, Nashville, TN (Poster)
 - Farinas AF, Manzanera IE, Pollins AC, Cardwell NL, Nuessenbaum ME, Does MD, Dortch RD, Thayer WP. MRI DTI Tractography Can Identify Early Partial Injuries in a Rat Sciatic Nerve Model. Military Health Systems Research Symposium, August 20-23, 2018, Kissimmee, FL (Podium)*

- Farinas AF, Manzanera IE, **Pollins AC**, Cardwell NL, Dortch RD, Thayer WP. Developing a Fraction Anisotropy Cutoff Value to Diagnose Recovery in Peripheral Nerve Injury. ASPN Annual Meeting, February 1-3, 2019, Palm Desert, CA. (Podium)
- Farinas A, Manzanera IE, **Pollins A**, Glassman G, Dortch RD, Thayer WP. Diffusion Tensor Imaging as a diagnostic tool to assess partial nerve laceration severity and recovery in a rat model ex vivo. ASPN Annual Meeting, January 10-12, 2020, Ft. Lauderdale, FL (ePoster).
- Manzanera IE, Farinas A, **Pollins A**, Thayer WP, Dortch RD. Assessment with Partial Peripheral Nerve Transection with Diffusion MRI. ISMRM Annual Meeting, April 17-20, 2020, Sydney, NSW, Australia (Poster), Meeting cancelled due to COVID-19.

▪ **Website(s) or other Internet site(s)**

Nothing to report.

▪ **Technologies or techniques**

Nothing to report.

▪ **Inventions, patent applications, and/or licenses**

Nothing to report.

▪ **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

▪ **What individuals have worked on the project?**

Name:	Wesley P. Thayer, M.D., Ph.D.
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-8160-9552 0000-0002-6512-6312
Nearest person month worked:	2
Contribution to Project:	As PI, Dr. Thayer supervised all activities associate with the work and reviewed all data. He critically evaluated, edited, and approved all data presentations, and generated all regulatory reports, including this one.

Name:	Richard D. Dortch, Ph.D.
Project Role:	Other (Collaborator)
Researcher Identifier (e.g. ORCID ID):	0000-0002-9978-2203
Nearest person month worked:	2
Contribution to Project:	As Collaborator and expert in MRI, Dr. Dortch supervised all MRI activities associated with the work and reviewed all data. He critically evaluated, edited, and approved all data presentations and manuscripts.

Name:	Nancy Cardwell, BS
Project Role:	Research Staff

<i>Researcher Identifier (e.g. ORCID ID):</i>	N/A
<i>Nearest person month worked:</i>	4
<i>Contribution to Project:</i>	<i>As research staff, Ms. Cardwell performed histology on animal specimens. Ms. Cardwell also aided in tracking progress of the study and managed supplies and resources. She critically evaluated, edited, and approved all data presentations.</i>

<i>Name:</i>	<i>Angel Farinas Chopite, M.D.</i>
<i>Project Role:</i>	<i>Research Fellow</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	0000-0002-9456-1444
<i>Nearest person month worked:</i>	7
<i>Contribution to Project:</i>	<i>As research fellow following Dr. Nussenbaum, Dr. Farinas was responsible for the animal work of Aim 1, Task 2. Dr. Farinas critically evaluated, edited, and approved all data presentations, and provided data for the regulatory reports. Dr. Farinas also attended conferences where he presented work from this funding.</i>

<i>Name:</i>	<i>Isaac Manzanera-Esteve, Ph.D.</i>
<i>Project Role:</i>	<i>Research Staff</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	0000-0002-9282-7026
<i>Nearest person month worked:</i>	5
<i>Contribution to Project:</i>	<i>As research staff, Dr. Manzanera-Esteve performed all the MRI scans of animal subjects and analyzed all the resulting data. Dr. Manzanera-Esteve critically evaluated, edited, and approved all data presentations, and provided data for the regulatory reports. He also provided assistance with the analysis of the human subject data. Dr. Manzanera-Esteve also attended conferences where he presented work from this funding.</i>

<i>Name:</i>	<i>Marlieke Nussenbaum, M.D.</i>
<i>Project Role:</i>	<i>Research Fellow</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	N/A
<i>Nearest person month worked:</i>	2
<i>Contribution to Project:</i>	<i>As research fellow at the start of the grant, Dr. Nussenbaum was responsible for the initial animal work and completed all animals in Aim 1, Task 1. Dr. Nussenbaum critically evaluated, edited, and approved all data presentations, and provided data for the initial regulatory reports.</i>

Name:	Alonda C. Pollins, MLI
Project Role:	Research Staff
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	As research staff, Ms. Pollins performed histology on animal specimens and analyzed all the resulting data. Ms. Pollins also aided in tracking progress of the study and managed supplies and resources. She critically evaluated, edited, and approved all data presentations, and provided data for the later regulatory reports, including this one.

Name:	Jun Yao, RN
Project Role:	Research Nurse
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	As research nurse, Ms. Yao worked to keep our clinical trial up-to-date. She attempted to get us back on track with recruitment and worked closely with our IRB to remain in compliance.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
Nothing to report.
- **What other organizations were involved as partners?**
 - **Organization Name:** Vanderbilt University (VU)
 - **Location of Organization:** Nashville, TN
 - **Partner's contribution to the project (identify one or more)**
 - **In-kind support** Partner makes software, computers, and equipment, available to project staff;
 - **Facilities** Vanderbilt University Institute for Imaging Science, where all MRI scans took place is housed here;
 - **Collaboration** Dr. Dortch and Dr. Does are both employees of VU and have lab space at VU;
 - **Personnel exchanges** Dr. Manzanera-Esteve started out as a post-doc under Dr. Dortch and eventually was promoted to Research Assistant Professor.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
Not applicable.
- **QUAD CHART:** See Below

9. APPENDICES:

MRI Diffusion Tensor Tractography to Track and Monitor Peripheral Nerve Recovery after Severe Crush or Cut/Repair Nerve Injury

W81XWH-16-1-0605 Log # MR 150075



PI: Wesley Thayer MD/PhD, Richard Dortch PhD Mark Does PhD Org: Vanderbilt University. Award Amount: \$1,500,00.00

Study/Product Aim(s)

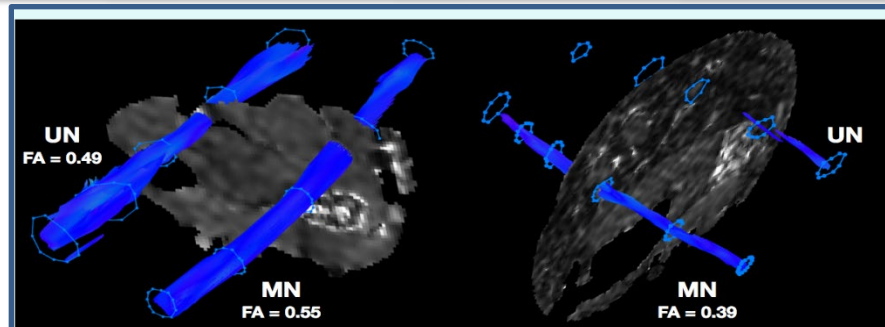
•Assess MRI/ DTI metrics to monitor and predict nerve regrowth following crush or cut with surgical repair. For patients with proximal nerve injury, this may allow early detection of failed nerve repairs or help determine the need for surgical intervention after severe crush injury.

•Aims:

- 1 Optimization of DTI metrics in animal models.
- 2 Implementation of DTI to monitor post nerve injury human clinical cases

Approach

DTI quantifies anisotropic diffusion behavior of water molecules in tissues with elongated cells (e.g., axons). In DTI, water diffusion is measured in multiple directions to obtain parameters that describe tissue structure and orientation at a microscopic level. In the animal model, we can detect gradations of injury and axonal continuity after microsurgical repair.



- MRI/ DTI metrics to monitor and predict nerve regrowth following crush or cut with surgical repair. For patients with proximal nerve injury, this may allow early detection of failed nerve repairs or help determine the need for surgical intervention after severe crush injury.
- Animal and human data acquisition completed. Findings published in

Accomplishment: Animal and human studies are completed w data analysis. DTI can be used to assess peripheral nerve recovery in animals and humans. 4 papers accepted including a Scientific Report article and a fifth article is submitted.

Timeline and Cost

Activities	CY	16	17	18	19	20
Optimize DTI in animal model						
DTI IRB approval (DOD)						
DTI eval of human nerve injury						
Data analysis / publication prep						
Estimated Budget (\$K)		\$125	\$500	\$500	\$375	

Goals/Milestones

CY16Goal – Optimize DTI protocol in animal nerve injury model
X Develop protocols and begin benchtop and in vivo testing
X Initiate DOD IRB process (complete)

CY17 Goals – Validate DTI metrics with in vivo models
X Complete protocol development
X DTI evaluation w of animal injury model (ended)
X Begin human testing

CY18 Goal – Animal and human testing (ended)
X Complete animal model experiments (ended)

CY19 Goal –
X Complete human case studies (ended)
X Data analysis and publication (4 accepted, one in process)

Comments/Challenges/Issues/Concerns

- Work is completed. Final publication submitted

Budget Expenditure to Date thru 9-30-20

Projected Expenditure: \$1,500,000

Updated: (3-28-21)

OPEN

Probabilistic Assessment of Nerve Regeneration with Diffusion MRI in Rat Models of Peripheral Nerve Trauma

Isaac V. Manzanera Esteve^{1,2}, Angel F. Farinas³, Alonda C. Pollins³, Marlieke E. Nussenbaum³, Nancy L. Cardwell³, Hakmook Kang⁴, Mark D. Does^{1,2,5}, Wesley P. Thayer^{3,5} & Richard D. Dortch^{1,2,5*}

Nerve regeneration after injury must occur in a timely fashion to restore function. Unfortunately, current methods (e.g., electrophysiology) provide limited information following trauma, resulting in delayed management and suboptimal outcomes. Herein, we evaluated the ability of diffusion MRI to monitor nerve regeneration after injury/repair. Sprague-Dawley rats were divided into three treatment groups (sham = 21, crush = 23, cut/repair = 19) and *ex vivo* diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI) was performed 1–12 weeks post-surgery. Behavioral data showed a distinction between crush and cut/repair nerves at 4 weeks. This was consistent with DTI, which found that thresholds based on the ratio of radial and axial diffusivities ($RD/AD = 0.40 \pm 0.02$) and fractional anisotropy ($FA = 0.53 \pm 0.01$) differentiated crush from cut/repair injuries. By the 12th week, cut/repair nerves whose behavioral data indicated a partial recovery were below the RD/AD threshold (and above the FA threshold), while nerves that did not recover were on the opposite side of each threshold. Additional morphometric analysis indicated that DTI-derived normalized scalar indices report on axon density (RD/AD : $r = -0.54$, $p < 1e-3$; FA : $r = 0.56$, $p < 1e-3$). Interestingly, higher-order DKI analyses did not improve our ability classify recovery. These findings suggest that DTI may provide promising biomarkers for distinguishing successful/unsuccessful nerve repairs and potentially identify cases that require reoperation.

Traumatic peripheral nerve injuries (TPNIs) are commonly caused by penetrating injuries, crush mechanisms, stretch, lacerations, and/or ischemia¹. Primary repair is recommended when nerve ends are severed without tension^{2–4}; however, approximately 40% of these surgeries fail and require a secondary surgical procedure to restore sensory and motor function⁵. After repair, nerve regeneration occurs at approximately 1 mm/day, which can result in a lengthy recovery when the injury site is not in close proximity to the neuromuscular junction⁶. Although electromyography and nerve conduction studies (NCS) are the current standards to assess nerve regeneration in extremities⁷, these methods are incapable of monitoring and quantifying nerve recovery until muscles are reinnervated. Thus, with no insight into the recovery process, physicians are forced to delay second interventions until the time at which axons should be reaching the muscular end plate (e.g., approximately 3 months for injuries at the mid-palm and 6 months for injuries at the wrist in adults)⁸, which increases the probability of irreversible muscular atrophy^{9,10}. Given these limitations, new methods are needed to provide an accurate evaluation of nerve regeneration throughout the recovery process to improve clinical decision-making and outcomes in patients.

Diffusion-weighted magnetic resonance imaging (MRI) probes tissue features at the microstructural level by measuring the effect of tissue barriers on the apparent diffusion of water molecules¹¹. In nerves, the ordered

¹Vanderbilt University Medical Center, Department Radiology and Radiological Sciences, Nashville, TN, USA.

²Vanderbilt University Medical Center, Institute of Imaging Science, Nashville, TN, USA. ³Vanderbilt University Medical Center, Department of Plastic Surgery, Nashville, TN, USA. ⁴Vanderbilt University Medical Center, Department of Biostatistics, Nashville, TN, USA. ⁵Vanderbilt University, Department of Biomedical Engineering, Nashville, TN, USA. *email: richard.dortch@vanderbilt.edu

arrangement of axons results in an apparent diffusion coefficient (ADC) that is lower perpendicular to axons than parallel to them^{12–14}. Diffusion tensor imaging (DTI) is a commonly used approach that measures diffusion along six or more directions to quantify indices that describe this diffusion anisotropy, including the mean diffusivity (MD, mean value across all directions), axial diffusivity (AD, diffusivity along axons), radial diffusivity (RD, diffusivity across axons), and fractional anisotropy (FA = 0–1, higher values indicate higher anisotropy). Higher-order diffusion kurtosis imaging (DKI) methods^{15–19} further quantify the effect of restricted diffusion within axons and/or heterogeneity (e.g., different intra/extra-axonal diffusivities), yielding measures of mean (MK, mean kurtosis across all directions), axial (AK, kurtosis along axons), and radial kurtosis (RK, kurtosis across axons) that are potentially more specific to axon microstructure^{20–23} at the cost of longer scan times (i.e., more diffusion directions and/or weightings than conventional DTI). For these kurtosis measures, larger values are indicative of increased non-Gaussian diffusion from tissue heterogeneity and/or restricted diffusion.

Based upon its sensitivity to nerve microstructure, diffusion MRI methods have been used to monitor nerve degeneration and/or regeneration in animal models^{24–28}. From these studies, it has been established that DTI metrics longitudinally track with electrodiagnostic and functional assessments of recovery. In addition, RD and FA values have been shown to correlate with behavior and histological measures of axon density during regeneration²⁶. We recently demonstrated that DTI indices also report on injury severity acutely after transection and surgical repair⁸ and these indices track with behavioral recovery over time²⁹. DKI indices may offer improved specificity to changes in nerve microstructure; however, the relationship between DKI parameters and peripheral nerve regeneration has yet to be validated. Nevertheless, recent work has indicated that nerve DKI is both feasible and sensitive to nerve injury³⁰.

While previous MRI studies have demonstrated the relationship between diffusion MRI indices in nerves and function or histology^{8,24,26,27}, no studies have evaluated the ability of diffusion MRI to stratify successful from unsuccessful repairs after TPNI. In this study, we propose a probabilistic model of nerve recovery based on multiple diffusion MRI parameters. To develop this model, we performed high-resolution *ex-vivo* DTI and DKI at various timepoints after *i)* injuries that are self-resolving (crush) and *ii)* injuries that show variable recovery (neurotmesis followed by surgical repair, i.e. cut/repair). Additional sham surgeries were performed for comparison. From these data, a probabilistic model of nerve recovery was developed and validated against behavioral and pathological findings. In addition, cut-off values that distinguish successful/unsuccessful repairs were determined, with the long-term goal of using these cut-off values to identify cases that require reoperation.

Results

Diffusion and kurtosis parameter maps. Figure 1 shows representative images and parameter maps from a distal slice in cut/repair nerves 12 weeks after repair for recovered (top panel) and non-recovered nerves (bottom panel) based on behavioral findings. Note the elevated diffusivities (AD, RD, and MD), reduced kurtosis indices (AK, RK, and MK), and reduced FA values in the non-recovered nerve relative to the recovered nerve. In addition, note the heterogeneity in parameter maps of the non-recovered nerve relative to the recovered nerve, which may be indicative of partial regeneration in this nerve.

Evolution of diffusion and kurtosis parameters following surgery. Figure 2 summarizes the evolution of the mean diffusion/kurtosis parameter across all distal slices for each cohort after surgery. Changes in diffusion parameters in the first two weeks can be linked to inflammatory processes, while the observed changes at 4 and 12 weeks can be linked to de/regeneration^{8,11,13,19,25}. With this in mind, RD values decreased in a similar fashion after surgery in crush and sham nerves, while a slower and less substantial decrease in RD was observed in cut/repair nerves ($p < 1e-3$ for 2, 4, and 12 weeks), which may be due to heterogeneity within in this cohort (i.e., not all cut/repair nerves recovered). In contrast, AD differed across all three cohorts. In particular, a significant decrease in AD was observed at two weeks after crush injury relative to both sham ($p < 1e-3$) and cut/repair cohorts ($p < 1e-3$), suggesting that AD and RD are sensitive to different pathological features during the recovery process. Because MD was formed from a weighted average of AD and RD, it did not provide unique information. The kurtosis parameters exhibited an inverse trend relative to each DTI parameter, although the temporal evolution may differ between diffusion and kurtosis parameters (e.g., RD reached a maximum at two weeks, while RK reached a minimum at four weeks in cut/repair nerves).

Probabilistic model of recovery based on diffusion and kurtosis parameters. In developing a probabilistic model of nerve recovery, we analyzed the ability of combinations of DTI and DKI parameters to differentiate between self-resolving crush injuries and partially recovering cut/repair nerve injuries. Of particular note, we analyzed slice-wise radial vs. axial parameters as shown in Fig. 3. During the first two weeks, neither DTI nor DKI parameters demonstrated a clear differentiation between injury types, likely due to the dominant effect of edema. By the fourth week however, RD vs. AD plots yielded non-overlapping clusters for each cohort. Note that this separation was not seen in the counterpart RK vs. AK plot; therefore, we limit our discussion below to RD vs. AD. The contrast observed in the RD vs. AD plot at four weeks is consistent with the longitudinal behavioral data in Fig. 4, which indicated that the crush nerves were fully recovered by four weeks while cut/repair nerves were not. Based upon this differentiation and the assumption that regeneration is complete 12 weeks after surgery, we predicted that the cut/repair nerves whose RD/AD values overlap with crush nerves at 12 were successful repairs, while those that did not overlap with were unsuccessful repairs.

To generate this predictor, the linear boundary between crush and cut/repair nerves was calculated for distal RD vs. AD values at four weeks using SVM analysis (see *Methods*). The decision boundary (RD/AD slope = 0.40 ± 0.02 , intercept = -0.03 ± 0.01) separated recovered nerves (crush) from non-recovered nerves (transected/repair) four weeks after injury. This decision boundary was then tested to the other distal timepoints (1, 2, and 12 weeks) as well as proximal nerve segments and sham nerves for all timepoints (1, 2, 4, and 12

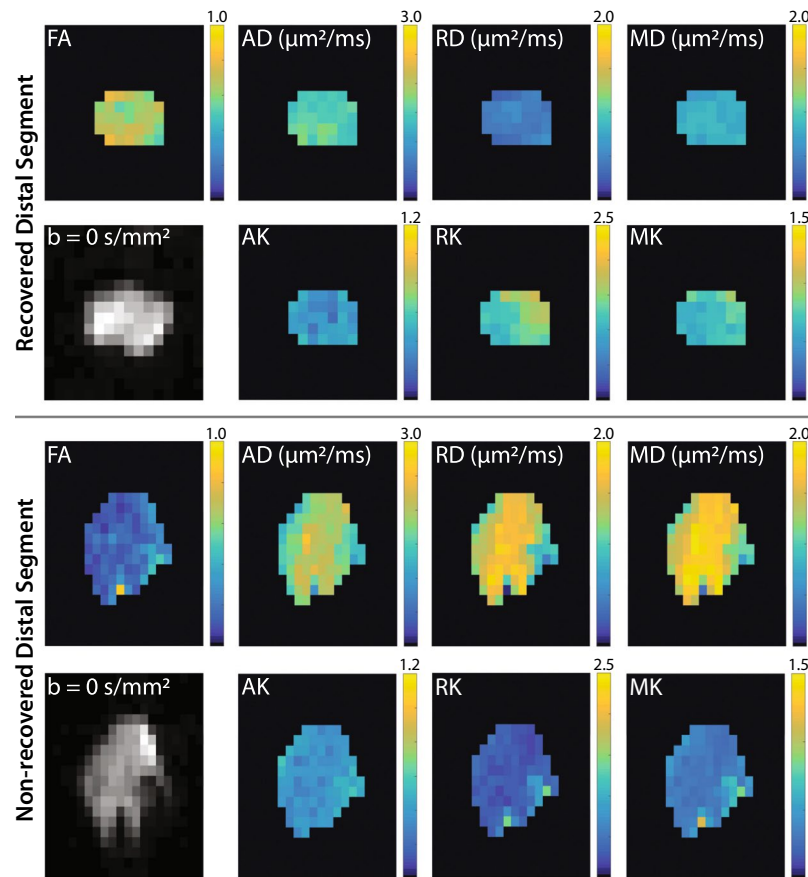


Figure 1. Representative distal DTI/DKI parameter maps from recovered (top panel) and non-recovered (bottom panel) cut/repair nerves 12 weeks after injury. Shown are maps of DTI (FA, AD, RD, and MD) and DKI (AK, RK, and MK) parameters along the non-diffusion weighted image ($b = 0 \text{ s/mm}^2$) from a single distal slice. Note the reduced FA (blue hues) and elevated diffusivities (yellow hues) in the non-recovered nerve relative the recovered nerve. In addition, note the reduce kurtosis measures (blue hues) in the non-recovered nerve.

weeks). Scatterplots of the results color-coded by probability of recovery (see *Methods*) can be seen in Fig. 5. Once again, distal segments of injured nerves (top row) were dominated by inflammation/edema in the first two weeks, resulting in low recovery probabilities. By the fourth week, behavioral data (Fig. 4) indicated that crush injuries were fully recovered, while cut/repair injuries were not yet recovered. This is reflected in Fig. 5 where recovery probabilities were above 50% for all crush injuries and below 50% for all cut/repair injuries. At 12 weeks, however, a subset of cut/repair nerves had a high probability of recovery, while other nerves had a low probability of recovery. Results from the proximal segments and sham nerves further validated this model, with increasing recovery probabilities as edema subsided and nearly full recovery by 4 and 12 weeks.

Correlations between model probabilities in distal crush and cut/repair nerves at 12 weeks and behavioral measures are shown in Fig. 6. Note the significant correlations between the model-derived probabilities and both SFI ($r = 0.93$, $p < 1e-3$) and FF ($r = 0.83$, $p < 1e-3$), which serves to validate this model.

Converting RD/AD to an FA threshold. Although the ratio RD/AD stratifies crush and cut/repair nerves at 4 weeks, it is not a conventionally reported DTI metric, which makes comparison of our findings herein with previous literature difficult. As a result, we transformed RD/AD to a corresponding FA cut-off using the following relationship³¹

$$FA = \frac{1 - \left(\frac{RD}{AD}\right)}{\sqrt{1 + 2\left(\frac{RD}{AD}\right)^2}}, \quad (1)$$

which assumes a cylindrically symmetric tensor. This indicates that a linear RD/AD boundary with zero offset can be represented by a unique FA threshold $= 0.53 \pm 0.01$. To test this value, Fig. 7 shows distal slice-wise FA profiles 12 weeks after injury for six samples stratified by behavioral recovery: two fully recovered crush nerves, two non-recovered cut/repair nerves, and two fully recovered cut nerves. It can be seen that FA values along the length of both crush nerves are considerably above the threshold, in agreement with the statement that all crush injuries fully recover. FA values for the distal regions of cut/repair nerve injuries that recovered were also above the FA

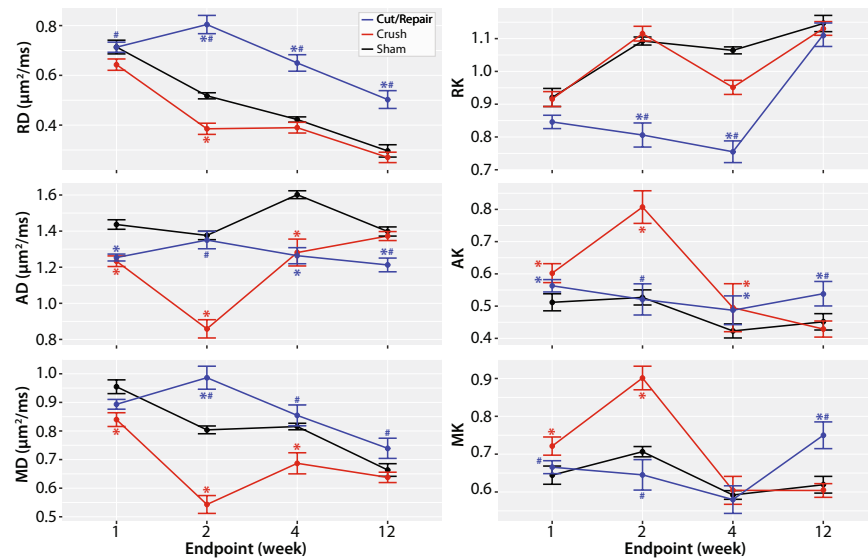


Figure 2. Evolution of diffusivities (left column) and kurtosis parameters (right column) after each injury/surgical intervention. Changes in parameters at 1 and 2 weeks are influenced by inflammation/edema, while changes at weeks 4 and 12 are indicative of de/regeneration. RD values decreased in a similar fashion after surgery in crush and sham nerves, while a slower and less substantial decrease in RD was observed in cut/repair nerves. In contrast, AD differed across all three cohorts, but were similar in crush and cut/repair nerves after four weeks. Finally, the kurtosis parameters exhibited a similar, but inverse, trend relative to each diffusion parameter. Significant differences at each timepoint are indicated as follows: cut/repair vs. sham = blue asterisk, crush vs. sham = red asterisk, cut/repair vs. crush = blue hashtag.

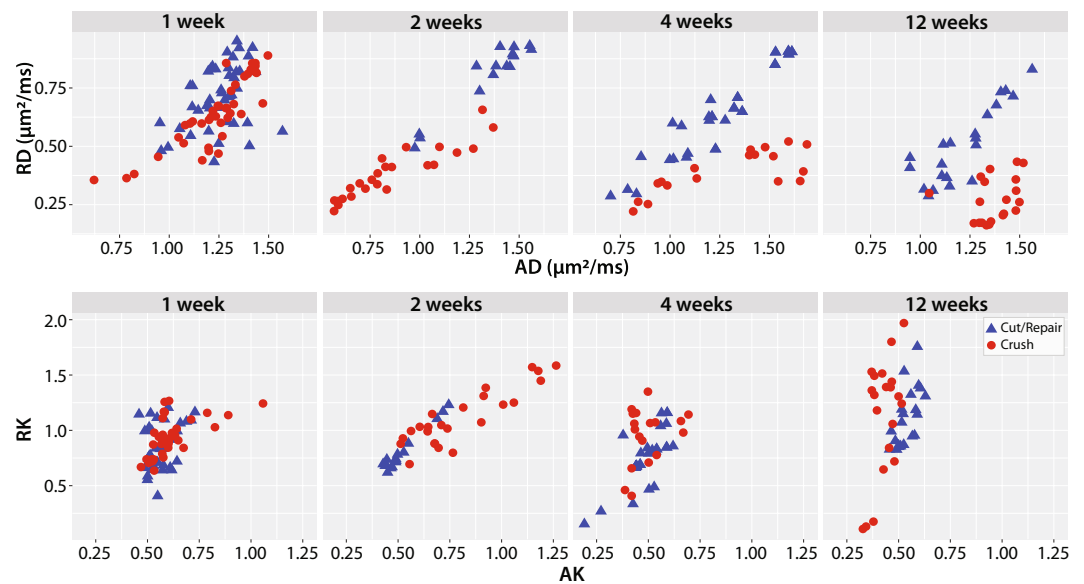


Figure 3. Scatterplots of distal radial vs. axial diffusivities (top panel) and kurtosis indices (bottom panel) for crush and cut/repair nerves at each time point after injury (left-to-right). Each point represents the mean cross-sectional value for a slice distal to the injury site.

threshold, while nerves with failed recoveries display values below this threshold. In addition, note the reduction in FA near the injury site for the crush and cut/repair nerves even 12 weeks after injury. This may be due to nerve sprouting and a reduction in coherence of axons that takes place at the injury site^{10,27,32}. In addition, since nerve growth is not homogeneous along the nerve, we can expect the density of fibers to decrease distal to the injury, which may be reflected by the reduced FA values in the most distal regions of the recovered cut/repair nerves.

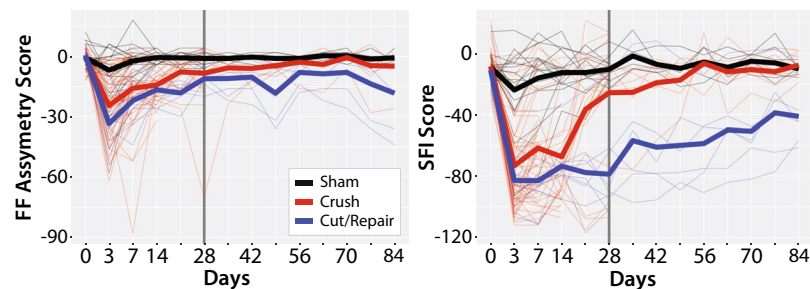


Figure 4. Longitudinal behavioral data after sham (black lines), crush (red lines) and cut/repair injuries (blue lines). Results are shown for both FF (left panel) and SFI (right panel). The longitudinal time course (from injury to euthanasia) for each individual animal is plotted with thin lines, while the mean time course within each cohort is plotted with thick lines. Note the sharp decay in FF and SFI for both cohorts immediately after surgery, with the largest contrast between injury types at 4 weeks (vertical black dashed line). These results indicate a faster behavioral recovery following crush relative to cut/repair injuries, with crush injuries nearly recovered by four weeks.

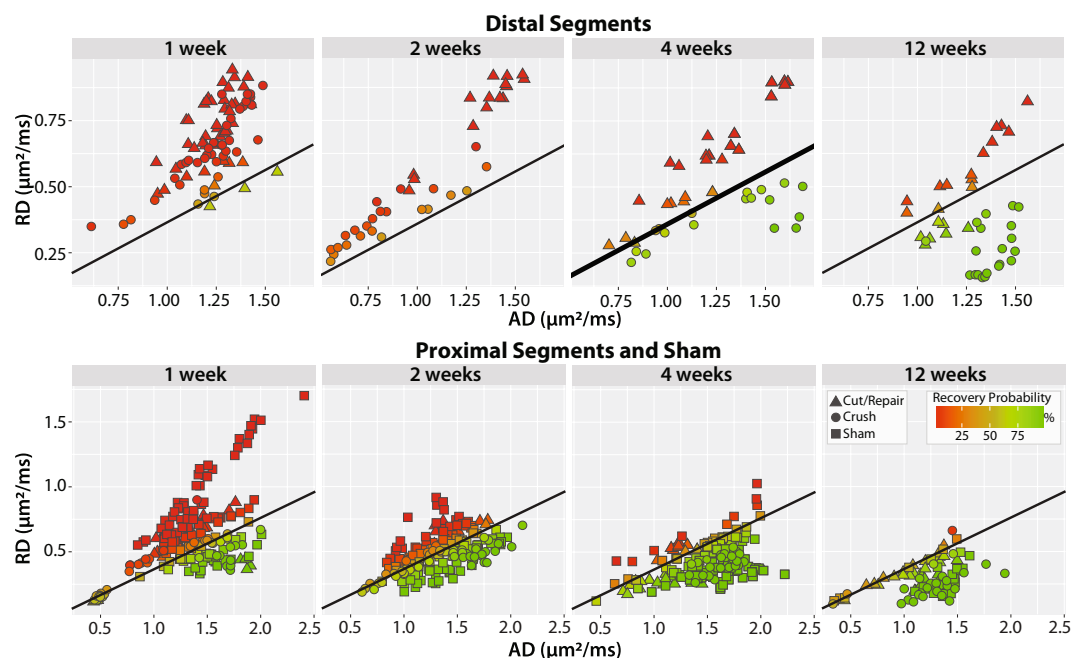


Figure 5. Scatterplots of radial vs. axial diffusivities for distal segments (top panel), proximal segments (bottom panel), and sham nerves (bottom panel). The SVM boundary was generated using the distal data at four weeks (thick black line) and tested using data from the other panels (thin black line). Each point represents the mean cross-sectional (or slice-wise) value. Recovery probabilities are color-coded from non-recovery (red, above the SVM boundary) to recovery (green, below the SVM boundary). Consistent with the behavioral findings in Fig. 4, the model indicates that crush injuries are recovered distally by the 4th week, while cut/repair nerves are not. By the 12th week, however, cut/repair nerves are found on both sides of the decision boundary (green/red triangles), indicating different probabilities of recovery. This model was further tested in sham nerves and the proximal regions of injured nerves (bottom panel), nearly all of which showed a high probability of recovery by the 4th week.

Comparison on DTI/DKI and histology. Figure 8 shows representative histological distal sections at 2, 4 and 12 weeks for the three cohorts. Correlations between DTI/DKI parameters in distal nerves and the resulting histologically-derived measures of axon density are shown in Fig. 9. Note the significant correlation between radial indices (RD: $r = -0.49$, $p < 1e-3$ and RK: $r = 0.40$, $p < 1e-2$) and axon density. In contrast, no correlation was observed between the axial indices (AD: $r = 0.10$, $p = 0.67$ and AK: $r = -0.32$, $p = 0.14$) and axon density. Consistent with our MRI findings in Fig. 4, DTI-derived normalized scalar indices (RD/AD: $r = -0.54$, $p < 1e-3$ and FA: $r = 0.56$, $p < 1e-3$) exhibited the strongest relationship with axon density. For comparison, proximal sections are shown in Supplemental Fig. 1. Note the absence of nerve degeneration in all of these sections, which suggests that we are detecting axonal sprouting with our distal DTI measures.

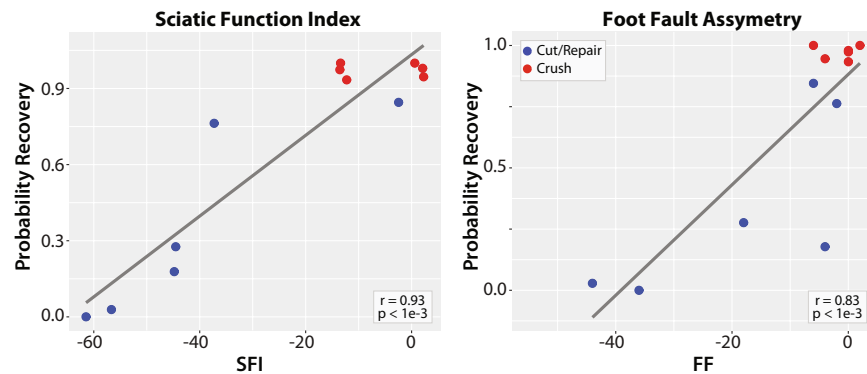


Figure 6. Linear correlations between DTI-derived probabilities of recovery and behavior (SFI and FF). Significant relationships were observed across distal crush (red) and cut/repair nerves (blue) at 12 weeks. The Pearson's correlation coefficient (r) and corresponding p -value is given in the white box of each panel.

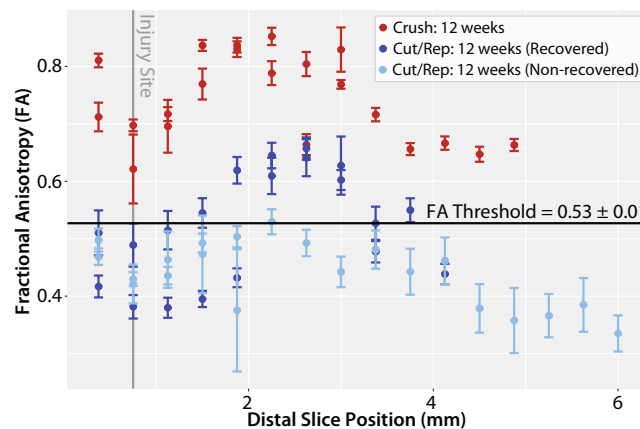


Figure 7. Slice-wise FA values (mean $\pm$ SEM) distal to the injury site (vertical gray line) for six nerve samples at 12 weeks after each intervention. Shown are representative results from two crush samples (red) along with the four cut/repair samples, two of which showed evidence of recovery via behavioral measures (dark blue) and two of which did not show evidence of recovery (light blue). In the recovered nerves, mean FA values were above the FA threshold = 0.53 ± 0.01 line for a majority of distal slices. In contrast, mean FA values were below this cut-off value for almost all distal slices in the non-recovered nerves.

Discussion

In this study, we built a DTI-based classifier that separates crush from cut/repair nerves 4 weeks after injury. Preliminary testing of this classifier 12 weeks after cut/repair injuries indicated that this classifier may also separate successful from unsuccessful nerve repairs. Consistent with previous studies²⁶, behavioral findings (Fig. 4) indicated that the crush nerves were fully recovered approximately 4 weeks after injury, while the cut/repair nerves did not substantially recover until after four weeks. In other words, a clear distinction between the behavioral recovery of crush and cut/repair nerves was observed at 4 weeks, which is consistent with the RD/AD measurements at the same timepoint in Fig. 3. Based upon this information, we developed a statistical model of recovery using distal RD/AD values 4 weeks after injury and surgical repair. As shown in Fig. 5, the linear model successfully classified the crush and cut/repair nerves at 4 weeks. By the 12th week, cut/repair nerves were found on both sides of the classification boundary, indicating different likelihoods of recovery. When further tested in sham nerves and the proximal regions of injured nerves, nearly all regions showed a high probability of recovery by the 4th week as expected. RD/AD values were then converted to more commonly reported FA values and an FA threshold was tabulated using Eq. (1). As shown in Fig. 5, the cut/repair nerves that recovered (based on behavioral findings) had slice-wise FA values that were above the FA threshold = 0.53 ± 0.01 for a majority of distal slices. In contrast, FA values were below this cut-off value for almost all distal slices in the non-recovered nerves. To validate this model histologically, light microscopy was performed in the distal regions of these same nerves and cross-sectional axon densities were manually estimated (Figs. 8 and 9). Consistent with previous work in crush injured nerves²⁶, both normalized scalar indices (RD/AD and FA) reported on changes in axon density during the recovery process for all three cohorts (sham, crush, and cut/repair).

In addition to nerve de/regeneration, the normalized scalar indices were influenced by edema within the first two weeks, which can be visualized as increased extra-cellular space in the light micrographs (Fig. 8). Because the model was trained using data after edema subsided (4 weeks), it does not consider edema effects present at

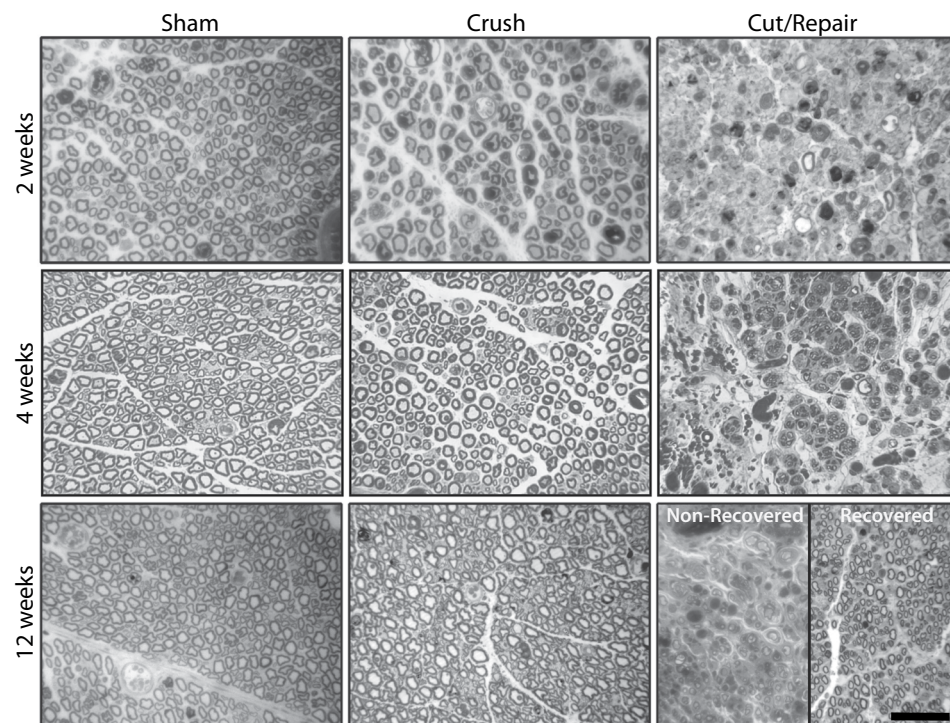


Figure 8. Representative distal toluidine blue stained sections at 2 (top panel), 4 (middle panel) and 12 weeks (bottom panel) after each injury type. In the sham groups (left column), there was little difference between the three times, except for some evidence of extra-cellular edema at 2 weeks. In contrast, crush nerves (middle column) shows indications of degeneration at 2 weeks and full recovery by 4 weeks. For the cut/repair group (right column), severe degeneration is observed at 2 and 4 weeks. By 12 weeks, a subset of cut/repair nerves showed little recovery (“non-recovered” panel), while another subset showed partial recovery (“recovered” panel). The scale bar in the bottom-right panel represents 50 μm .

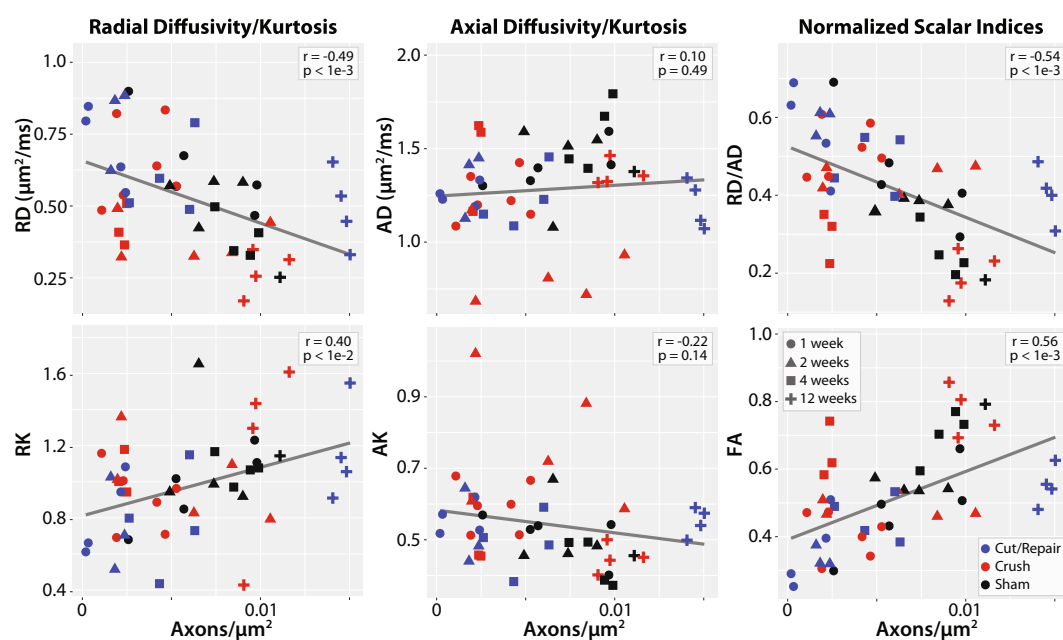


Figure 9. Linear correlations between DTI/DKI parameters and histologically-estimated cross-sectional axon densities. Results are shown for all timepoints (encoded via marker shape) and injury type (encoded via marker color). Significant correlations were observed between radial parameters (RD and RK) and axon density (left column), while the relationship between axial parameters (AD and AK) and axon density (middle column) was not significant. The normalized scalar indices from DTI (right column, RD/AD and FA) demonstrated the strongest relationships with axon density. The Pearson's correlation coefficient (r) and corresponding p -value is given in the white box of each panel.

Timepoints	Injury Types		
	Sham	Crush	Cut/Repair
1 week	6	5	5
2 weeks	6	6	3
4 weeks	6	6	5
12 weeks	3	6	6
Total	21	23	19

Table 1. Number of rats per injury cohort and timepoint.

1–2 weeks (Fig. 5). This limitation was further verified using data from sham nerves and the proximal regions of crush nerves at 1–2 weeks. Given this limitation, alternate models based on other diffusion parameters may be necessary to classify nerve recovery early after injury. For example, previous work^{33–35} has shown that axial diffusion parameters are primarily sensitive to axonal degeneration within the first few weeks after injury, which is consistent with the observed decrease in AD (and increase in AK) in crush nerves at 2 weeks (Fig. 2). In addition, diffusion models that account for the effects of edema¹² or filter out signal contributions from the extra-cellular space³⁶ may improve predictions of nerve recovery within the first few weeks.

One interesting finding of this study was that conventional DTI, without the need for a higher-order DKI model, was able to differentiate recovered from non-recovered nerves following injury and repair. Previous work in the central nervous system^{20–23} suggested that DKI indices may provide a more specific assay of axon microstructure than DTI, which is in conflict with our findings in peripheral nerves. These differences may be attributed in part to the different b-values (2000 and 4000 s/mm²) used herein, which were empirically determined to account for the decreased diffusivity caused by fixation³⁷ and temperature (bore temperature $\approx 20^\circ\text{C}$). Perhaps more significantly, axon diameters in peripheral nerves ($\approx 1\text{--}15\mu\text{m}$)³⁸ are nearly an order of magnitude larger than in the central nervous system, suggesting that longer diffusion times ($\Delta = 12\text{ ms}$ for the current study) may be required for optimal sensitivity to diffusion kurtosis in the presence of these larger axons³⁹. For example, axons are often modeled using the zero-radius approximation, which assumes $\Delta \gg R^2/D_a$, where R is the radius of the axon and $D_a \approx 1\mu\text{m}^2/\text{ms}$ is the free intra-axonal diffusivity⁴⁰. For white matter data acquired on a clinical system ($\Delta \approx 50\text{ ms}$, $R \approx 1\mu\text{m}$), the zero radius-approximation is valid. In contrast, this assumption is no longer valid for peripheral nerve data acquired herein ($\Delta = 12\text{ ms}$, $R \approx 5\mu\text{m}$). Given these differences, additional studies on the relative effects of restricted diffusion within axons and heterogeneity (i.e., differences in intra/extra-axonal diffusivities) on DKI parameters in nerves are warranted. Nevertheless, the ability to predict recovery via DTI is beneficial for clinical translation, as DKI requires longer scans than conventional DTI.

Although promising, two additional limitations of this study should be addressed in future work. First, *ex vivo* MRI was performed in fixed rather than fresh tissues due to the long scan times required for high-resolution MRI. Because fixation affects diffusivities, one cannot directly translate the absolute diffusivities reported herein to fresh tissues. Fortunately, previous studies³⁷ have indicated that normalized scalar indices (RD/AD and FA) are equivalent in fresh and fixed tissues. This suggests that the model proposed herein may be applicable *in vivo*, although additional studies are required to confirm this. Second, the current study investigated a cross-section of samples at several timepoints (1, 2, 4, and 12). Future studies should focus on (i) additional timepoints between 4–12 weeks to determine how early our model can stratify recovered from non-recovered nerves and (ii) longitudinal *in vivo* measurements to determine how model predictions relate to outcomes in the same nerve.

In conclusion, normalized scalar indices (RD/AD and FA) derived from high-resolution *ex vivo* DTI of rat sciatic nerve were found to differentiate crush (fully recovered) from cut/repair (partially recovered) injuries once the edema had subsided at 4 weeks post-injury. In addition, these indices related to behavioral recovery and histological measurements of axon density. Interestingly, higher order DKI models, which require longer scan times than DTI, did not improve the sensitivity of the model to regeneration. Currently, nerve recovery after surgical intervention requires a “wait and watch” approach due to the absence of a reliable noninvasive biomarker, which increases the likelihood of poor outcomes. The studies herein suggest that RD/AD and FA may be promising biomarkers for distinguishing successful/unsuccessful repairs earlier than currently possible and potentially identify cases that require reoperation, although additional *in vivo* longitudinal studies are required to determine how model predictions relate to long-term outcomes.

Methods

Ethics approval. All animal procedures were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee, under the Guide for Care and Use of Laboratory Animals to minimize pain and suffering.

Experimental design. Sixty-three Sprague-Dawley rats were used as experimental animals. Each rat was randomly assigned to a traumatic peripheral nerve injury cohort (sham, crush, or cut/repair) and timepoint (1, 2, 4, or 12 weeks) as detailed in Table 1. Behavioral tests were performed before surgical intervention, three days after intervention, and then weekly until each animal's endpoint to evaluate recovery following each intervention. Note that all nerve repairs were performed using the same surgical procedures. Based upon previous literature⁵, we expected approximately half of these repairs to be successful, which is consistent with the findings presented herein. Behavioral measurements included foot fault (FF) asymmetry score and sciatic function index (SFI), as described in previous studies⁴¹. Each animal was euthanized at its assigned timepoint to perform *ex-vivo* MRI scanning and histology.

Animal surgeries. Induction and general anesthesia were performed with a dose of 3 mL/min of 2% Isoflurane, and care was taken the potential for hypothermia. A 3-cm skin incision was then made from below the ischial notch parallel the longitudinal axis of the hind leg. Dissection was done by planes to free the sciatic nerve proximally and distally up to its trifurcation. Within this nerve segment and 1 cm proximal to the trifurcation, each animal received one of the following: application of a Hemostat for 10 seconds (crush, $n = 23$), full transection with immediate repair (cut/repair, $n = 19$), or no intervention (sham, $n = 21$). Repair surgery consisted of an end-to-end fashion with interrupted epineurial 9-0 nylon sutures (Ethicon, Somerville, NJ). Wounds were closed in 2 layers using 5-0 Monocryl suture (Ethicon, Somerville, NJ). After surgery, animals were carefully monitored for any adverse anesthetic effect and provided with daily injections of ketoprofen (5 mg/kg) for 3 days post-operatively. At each animal's endpoint, euthanasia was performed with an intracardiac dose of 120 mg/kg of Euthasol (Virbac AH, Fort Worth, Texas), and nerves were harvested.

Tissue sample preparation. Harvested nerves were fixed by immersion in 3% glutaraldehyde/2% paraformaldehyde, washed for a minimum of 1 week in phosphate-buffered saline (PBS) to remove excess fixative, then immersed in 1 mM Gd-DTPA (Magnevist; Berlex, Montville, NJ) at 4 °C for at least 36 hours to reduce spin-lattice relaxation times and corresponding scan times. Samples were trimmed to approximately 1 cm in length with crush and cut/repair regions at the center of each segment. Finally, nerves were placed in 1.75-mm glass capillary tubes filled with a perfluorocarbon solution (Fomblin; Solvay, Thorofare, NJ) to prevent tissue drying without contributing to the proton MRI signal.

MRI protocol. To improve throughput, groups of six nerves were arranged in a hexagonal pattern and scanned simultaneously. Diffusion-weighted MRI data were acquired at bore temperature ($\approx 20^\circ\text{C}$) using a 7-T, 16 cm bore Bruker Biospec console (Rheinstetten, Germany) and a 25-mm quadrature radio-frequency coil (Doty Scientific, Columbia, SC) for transmission and reception. Images were acquired with a three-dimensional diffusion-weighted spin-echo sequence and the following parameters: field-of-view = $60 \times 60 \times 160 \text{ mm}^3$, resolution = $125 \times 125 \times 372 \mu\text{m}^3$, TE/TR = 22/425 ms, gradient pulse duration/diffusion time (δ/Δ) = 4/12 ms, b-values = 2000 and 4000 s/mm^2 , 20 diffusion directions, number of averaged excitations = 2, and scan time = 7 hours and 40 minutes for each b-value.

MRI analysis. Diffusion and kurtosis tensors were estimated on voxel-wise basis using a weighted linear least-squares estimation¹⁸ in MATLAB 2017b (Mathworks, Natick, MA). The following indices were estimated from the diffusion tensor: FA, MD, RD, AD. From the kurtosis tensor, we additionally estimated MK, AK, and RK. Each slice was manually classified as proximal to injury, within zone of injury, or distal to injury; and regions-of-interest (ROI) were drawn manually in each nerve to calculate the mean slice-wise diffusion parameters.

Histology. Consecutively after MRI scans, samples were fixed in 3% glutaraldehyde/2% paraformaldehyde, counterstained with 1% OsO₄ solution, dehydrated in increasing concentrations of ethanol, and embedded in resin. Sections of 1 μm were obtained from the center of the distal segment then stained with 1% toluidine blue for examination with light microscopy (Olympus Vanox-T AH-2). Two samples suffered from image quality issues related to fixation, embedding, an/or sectioning difficulties; therefore, these examples were excluded from subsequent analyses. Axon counts and cross-sectional areas were manually measured at 40x magnification using Image Pro Plus 7.0 (Media Cybernetics, Bethesda, MD), and axon density was estimated from the number of axons/cross-sectional area. This process was repeated in three randomly selected areas per sample and the mean axon density was reported.

Statistical analysis. All statistical analyses were performed R version 3.3.2. For each timepoint and DTI/DKI parameter, pairwise differences between cohorts (sham, crush, cut/repair) were assessed using an unpaired student's t-test. Correlations between DTI/DKI parameters and histologically-derived axon densities were quantified via Pearson's correlation coefficient. Samples with $\text{RD} > 1 \mu\text{m}^2/\text{ms}$, $\text{RK} < 0$, or $\text{RK} > 2$ were deemed outliers and excluded from this analysis^{20,42–44}. All p-values were adjusted for the effect of multiple comparisons using a false discovery rate approach⁴⁵.

A linear classifier for successful versus unsuccessful regenerations was generated for RD/AD values, which showed a clear separation between crush and cut/repair cohorts at 4 weeks after injury/repair. This linear classifier was generated via a support vector machine (SVM library e1071), and uncertainties in the classification boundary were calculated via Bootstrap. This boundary was then tested using distal RD/AD values at the other timepoints (1, 2, and 12 weeks). Additional testing was performed by applying the classification boundary to RD/AD values from sham nerves and the proximal segments of crush and cut/repair nerves at all timepoints (1, 2, 4, and 12 weeks). A *posteriori* classification probabilities were then estimated using an improved implementation of Platt's approach^{46,47}. This approach was employed rather than a direct classification using logistic regression because the cohorts were fully separable in the training data (distal RD/AD values at 4 weeks), which results in a degenerate solution when logistic regression is employed for classification purposes. To further validate the model, correlations between estimated probabilities at 12 weeks and disability scores were quantified using Pearson's correlation coefficient.

Data availability

The data related to the current study will be made available from the corresponding author upon reasonable request.

Received: 8 April 2019; Accepted: 5 December 2019;

Published online: 23 December 2019

References

1. Taylor, C. A., Braza, D., Rice, J. B. & Dillingham, T. The Incidence of Peripheral Nerve Injury in Extremity. *Trauma. Am. J. Phys. Med. Rehabil.* **87**, 381–385 (2008).
2. Moore, A. M., Wagner, I. J. & Fox, I. K. Principles of nerve repair in complex wounds of the upper extremity. *Semin. Plast. Surg.* **29**, 40–47 (2015).
3. Castillo-Galván, M. L., Martínez-Ruiz, F. M., de la Garza-Castro, O., Elizondo-Omaña, R. E. & Guzmán-López, S. Study of peripheral nerve injury in trauma patients. *Gac. Med. Mex.* **150**, 527–532 (2014).
4. Li, A. *et al.* A bioengineered peripheral nerve construct using aligned peptide amphiphile nanofibers. *Biomaterials* **35**, 8780–8790 (2014).
5. Vastamaki, M., Kallio, P. & Solonen, K. The Results of secondary microsurgical repair of ulnar nerve injury. *J. Hand Surg. Am.* **18B**, 323–326 (1993).
6. Ovalle, F. *et al.* A simple technique for augmentation of axonal ingrowth into chondroitinase-treated acellular nerve grafts using nerve growth factor. *Ann. Plast. Surg.* **68**, 518–524 (2012).
7. Han, D., Lu, J., Xu, L. & Xu, J. Comparison of two electrophysiological methods for the assessment of progress in a rat model of nerve repair. *Int. J. Clin. Exp. Med.* **8**, 2392–2398 (2015).
8. Boyer, R. B. *et al.* 4.7-T diffusion tensor imaging of acute traumatic peripheral nerve injury. *Neurosurg Focus* **39**, 1–9 (2015).
9. Weng, J., Zhang, P., Yin, X. & Jiang, B. The Whole Transcriptome Involved in Denervated Muscle Atrophy Following Peripheral Nerve Injury. *Front. Mol. Neurosci.* **11**, 1–15 (2018).
10. Campbell, W. W. Evaluation and management of peripheral nerve injury. *Clin. Neurophysiol.* **119**, 1951–1965 (2008).
11. Beaulieu, C. The basis of anisotropic water diffusion in the nervous system - A technical review. *NMR Biomed.* **15**, 435–455 (2002).
12. Pasternak, O., Sochen, N., Gur, Y., Intrator, N. & Assaf, Y. Free water elimination and mapping from diffusion MRI. *Magn. Reson. Med.* **62**, 717–730 (2009).
13. Alexander, A. L., Lee, J. E., Lazar, M. & Field, A. S. Diffusion Tensor Imaging of the Brain. *Neurotherapeutics* **4**, 316–329 (2008).
14. Mori, S. & Zhang, J. Principles of Diffusion Tensor Imaging and Its Applications to Basic Neuroscience Research. *Neuron* **51**, 527–539 (2006).
15. Steven, A. J., Zhuo, J. & Melhem, E. R. Diffusion kurtosis imaging: An emerging technique for evaluating the microstructural environment of the brain. *Am. J. Roentgenol.* **202**, 26–33 (2014).
16. Shahim, P., Holleran, L., Kim, J. H. & Brody, D. L. Test-retest reliability of high spatial resolution diffusion tensor and diffusion kurtosis imaging. *Sci. Rep.* 1–14, <https://doi.org/10.1038/s41598-017-11747-3> (2017).
17. Falangola, M. F. *et al.* Age-related non-Gaussian diffusion patterns in the prefrontal brain. *J. Magn. Reson. Imaging* **28**, 1345–1350 (2008).
18. Veraart, J., Sijbers, J., Sunaert, S., Leemans, A. & Jeurissen, B. Weighted linear least squares estimation of diffusion MRI parameters: Strengths, limitations, and pitfalls. *Neuroimage* **81**, 335–346 (2013).
19. Wu, E. X. & Cheung, M. M. MR diffusion kurtosis imaging for neural tissue characterization. *NMR Biomed.* 836–848, <https://doi.org/10.1002/nbm.1506> (2010).
20. Cheung, M. M. *et al.* Does diffusion kurtosis imaging lead to better neural tissue characterization? A rodent brain maturation study. *Neuroimage* **45**, 386–392 (2009).
21. Helpert, J. *et al.* Preliminary Evidence of Altered Gray and White Matter Microstructural Development in the Frontal Lobe of Adolescents With Attention-Deficit Hyperactivity Disorder: A Diffusional Kurtosis Imaging Study. *J. Magn. Reson. Imaging* **33**, 17–23 (2011).
22. Lee, C.-Y. *et al.* Microstructural integrity of early- versus late-myelinating white matter tracts in medial temporal lobe epilepsy. *Epilepsia* **54**, 1801–1809 (2013).
23. Kelm, N. D. *et al.* Evaluation of Diffusion Kurtosis Imaging in *Ex Vivo* Hypomyelinated Mouse Brains. *Neuroimage* **91**, 165–171 (2015).
24. Stanis, G. J., Midha, R., Munro, C. A. & Henkelman, R. M. MR properties of rat sciatic nerve following trauma. *Magn. Reson. Med.* **45**, 415–420 (2001).
25. Beaulieu, C., Does, M. D., Snyder, R. E. & Allen, P. S. Changes in water diffusion due to Wallerian degeneration in peripheral nerve. *Magn. Reson. Med.* **36**, 627–631 (1996).
26. Takagi, T. *et al.* Visualization of peripheral nerve degeneration and regeneration: Monitoring with diffusion tensor tractography. *Neuroimage* **44**, 884–892 (2009).
27. Morisaki, S. *et al.* *In vivo* assessment of peripheral nerve regeneration by diffusion tensor imaging. *J. Magn. Reson. Imaging* **33**, 535–542 (2011).
28. Lehmann, H. C., Zhang, J., Mori, S. & Sheikh, K. A. Diffusion tensor imaging to assess axonal regeneration in peripheral nerves. *Exp. Neurol.* **223**, 238–244 (2010).
29. Nussenbaum, M. *et al.* Using DTI MRI to Predict Peripheral Nerve Recovery in a Rat Sciatic Nerve Model. In *American Society for Peripheral Nerve* (2018).
30. Andersson, G., Orädd, G., Sultan, F. & Novikov, L. N. *In vivo* Diffusion Tensor Imaging, Diffusion Kurtosis Imaging, and Tractography of a Sciatic Nerve Injury Model in Rat at 9.4T. *Sci. Rep.* <https://doi.org/10.1038/s41598-018-30961-1> (2018).
31. Hasan, K. M. & Narayana, P. A. Retrospective measurement of the diffusion tensor eigenvalues from diffusion anisotropy and mean diffusivity in DTI. *Magn. Reson. Med.* **56**, 130–137 (2006).
32. Jeon, T., Fung, M. M., Koch, K. M., Tan, E. T. & Sneag, D. B. Peripheral nerve diffusion tensor imaging: Overview, pitfalls, and future directions. *J. Magn. Reson. Imaging* **47**, 1171–1189 (2018).
33. Sun, S. W. *et al.* Noninvasive detection of cuprizone induced axonal damage and demyelination in the mouse corpus callosum. *Magn. Reson. Med.* **55**, 302–308 (2006).
34. Sun, S.-W., Liang, H.-F., Cross, A. H. & Song, S.-K. Evolving Wallerian degeneration after transient retinal ischemia in mice characterized by diffusion tensor imaging. *Neuroimage* **40**, 1–10 (2008).
35. Song, S. K. *et al.* Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage* **20**, 1714–1722 (2003).
36. Budde, M. D., Skinner, N. P., Muftuler, L. T., Schmit, B. D. & Kurland, S. N. Optimizing Filter-Probe Diffusion Weighting in the Rat Spinal Cord for Human Translation. *Front. Neurosci.* **11**, 706 (2017).
37. Sun, S.-W., Neil, J. J. & Song, S.-K. Relative indices of water diffusion anisotropy are equivalent in live and formalin-fixed mouse brains. *Magn. Reson. Med.* **50**, 743–748 (2003).
38. Gillespie, M. J. & Stein, R. B. The relationship between axon diameter, myelin thickness and conduction velocity during atrophy of mammalian peripheral nerves. *Brain Res.* **259**, 41–56 (1983).
39. Jespersen, S. N., Olesen, J. L., Hansen, B. & Shemesh, N. Diffusion time dependence of microstructural parameters in fixed spinal cord. *Neuroimage* **182**, 329–342 (2018).

40. Fieremans, E., Jensen, J. H. & Helpert, J. A. White matter characterization with diffusional kurtosis imaging. *Neuroimage* **58**, 177–188 (2011).
41. Nguyen, L. *et al.* Engineered Porcine-derived Urinary Bladder Matrix Conduits as a Novel Scaffold for Peripheral Nerve Regeneration. *Ann Plast Surg.* **78**, 328–334 (2017).
42. Hui, E. S., Cheung, M. M., Qi, L. & Wu, E. X. Towards better MR characterization of neural tissues using directional diffusion kurtosis analysis. *Neuroimage* **42**, 122–134 (2008).
43. Hansen, B., Shemesh, N. & Jespersen, S. N. Fast imaging of mean, axial and radial diffusion kurtosis. *Neuroimage* **142**, 381–393 (2016).
44. Jensen, J. & Helpert, J. MRI Quantification of Non-Gaussian Water Diffusion by Kurtosis Analysis. *NMR Biomed.* **23**, 698–710 (2010).
45. Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A practical and Powerful Approach to Multiple. *Testing. J. R. Stat. Soc. Ser. B* **57**, 289–300 (1995).
46. Karatzoglou, A., Meyer, D. & Hornik, K. Support Vector Algorithm in R. *J. Stat. Softw.* **15**, 1–28 (2006).
47. Hornik, K., Weingessel, A., Leisch, F. & Davidmeyer-projectorg, M. D. M. *Package 'e1071'* (2018).

Acknowledgements

This work was supported by NIH/NINDS R01 NS97821 (RDD) and U.S. Army Medical Research and Materiel Command, Contract Number W81XWH-15-JPC-8/CRM RP-NMSIRA, Grant Number: MR150075 (WPT).

Author contributions

I.V.M.E.: development of MRI scan protocols, collection of MRI data, data/statistical analysis, figure preparation, manuscript preparation. A.F.F. and A.C.P.: behavioral and histological data collection, figure preparation, manuscript preparation. M.E.N.: performed all surgical procedures, manuscript review/editing. N.L.C.: supervision/care for animal models, manuscript review/editing. H.K.: data/statistical analysis, figure preparation, manuscript review/editing. M.D.D., W.P.T. and R.D.D.: development of study design, manuscript review/editing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41598-019-56215-2>.

Correspondence and requests for materials should be addressed to R.D.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019

Diffusion Tensor Tractography Visualizes Partial Nerve Laceration Severity as Early as 1 Week After Surgical Repair in a Rat Model Ex Vivo

Angel F. Farinas, MD*; Isaac V. Manzanera Esteve, PhD†§; Alonda C. Pollins, MLI*; Nancy L. Cardwell, BS*; Mark D. Does, PhD†‡§; Richard D. Dortch, PhD†‡§; Wesley P. Thayer, MD, PhD*†

ABSTRACT Background: Previous studies in our laboratory have demonstrated that a magnetic resonance imaging method called diffusion tensor imaging (DTI) can differentiate between crush and complete transection peripheral nerve injuries in a rat model ex vivo. DTI measures the directionally dependent effect of tissue barriers on the random diffusion of water molecules. In ordered tissues such as nerves, this information can be used to reconstruct the primary direction of diffusion along fiber tracts, which may provide information on fiber tract continuity after nerve injury and surgical repair. Methods: Sprague-Dawley rats were treated with different degrees of partial transection of the sciatic nerve followed by immediate repair and euthanized after 1 week of recovery. Nerves were then harvested, fixed, and scanned with a 7 Tesla magnetic resonance imaging to obtain DTI and fiber tractography in each sample. Additional behavioral (sciatic function index, foot fault asymmetry) and histological (Toluidine blue staining) assessments were performed for validation. Results: Tractography yielded a visual representation of the degree of injury that correlated with behavioral and histological evaluations. Conclusions: DTI tractography is a noninvasive tool that can yield a visual representation of a partial nerve transection as early as 1 week after surgical repair.

INTRODUCTION

During the period from World War II to the Global War Against Terrorism, mortality has decreased from 30 to 10% because of improved protective technology and more effective field evacuations.¹ Current procedures to preserve life, as well as limb, are applied immediately at the battlefield, helping to delay definitive treatment that could be miles away.² Improving combat survival has subsequently increased the number of complex wounds in which peripheral nerve morbidity is high.³ In Operation Iraqi Freedom and Operation Enduring Freedom only, more than half of the injuries involved extremities.⁴ Injuries to the extremities is the most common cause of disability after a combat wound, and disability involving peripheral nerve injuries can be as high as 70%.⁵ Some authors

have described that approximately 70% of the traumatic nerve injuries were caused by an explosion.^{3,5} In the upper extremity, the radial nerve is the most common culprit of disability in a wounded soldier, but the ulnar nerve at the level of the forearm is most common nerve injured.^{3,5} Disability can be defined as loss of function, military discharge, or chronic pain.³

According to Seddon, nerve injury can be classified depending on the degree of injury⁶ including compression with or without demyelination (neurapraxia), axon transection with remaining layers intact (axonotmesis), and complete transection (neurotmesis).⁶ Sunderland revised this classification into five degrees. The first- and second-degree injuries correlated with neurapraxia and axonotmesis, respectively. Sunderland also included two more degrees of injury dependent on if the endoneurium (third degree) or perineurium (fourth degree) was involved. Finally, fifth degree represent complete transection when all layers are injured, which is equivalent to Seddon's neurotmesis.⁶ In a study done by Birch et al.,³ where a total of 261 peripheral nerve injuries caused in Iraq and Afghanistan's warfare were observed, 45% were neurapraxia, 35% were axonotmesis, and 20% were neurotmesis, distributed equally between the upper and lower extremities.

The vast majority of experience with peripheral nerve repair comes from the military arena, mainly because of the caliber of the weapons involved in these injuries.⁵ The incidence of traumatic peripheral nerve injuries after Iraq's and Afghanistan's conflicts was 8%.³ But peripheral injuries are not exclusive of wartime; this morbidity is also encountered in the civilian hospitals as well. Each year, approximately 800,000 patients seen in trauma centers with some sort of peripheral nerve injury.⁷

*Department of Plastic Surgery, Vanderbilt University Medical Center, 1161 21st Ave S, MCN D4207, Nashville, TN 37232-2345

†Department of Biomedical Engineering, Vanderbilt University, 2301 Vanderbilt Place PMB 351631, Nashville, TN 37235-1631

‡Department Radiology and Radiological Sciences, Vanderbilt University Medical Center, 1161 21st Ave S, MCN CC-1121, Nashville, TN 37232-2345

§The Department is the Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Vanderbilt University, Institute of Imaging Science, 1161 21st Ave S, MCN AA-1105, Nashville, TN 37232-2345

Presented as oral talk at the 2018 Military Health Science and Research Symposium August 20–24, Kissimmee, FL; Abstract Number: MHSRS-18-1268.

This work was supported by U.S. Army Medical Research and Materiel Command, Contract Number W81XWH-15-JPC-8/CRMNP-NMSIRA, Grant number: MR150075. Additional support provided by NIH/NINDS R01 NS97821 (RDD).

doi:10.1093/milmed/usz360

© Association of Military Surgeons of the United States 2020. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Management of a peripheral nerve injury differs from the community to the combat zone. In the civilian setting, depending on the nature of the injury and concomitant injuries, these can be repaired immediately or delayed.⁸ Immediate repair after injury has surgical advantages including avoiding edema and scar tissue. Additionally, the topographic alignment with intraoperative stimulation can be useful in complex wounds.⁸ Conversely, in war periods, when a soldier suffers a peripheral nerve injury, they undergo damage control, serial debridement/decontamination, temporary bone stabilization, and plan for final repair and soft tissue coverage on a posterior date.² All combat wounds are considered contaminated with a high incidence of osteomyelitis if closed by primary intention.³ Nerve repair is mostly achieved in a delayed fashion because of the heavily contaminated wounds.² Segmental nerve loss typically treated with an autograft can be troublesome because of limited sources of donor tissue secondary to polytrauma and limb amputations.² At the time of formal neurotaphy, it is recommended to perform a nerve transfer because of the uncertain patient follow-up.²

Currently, clinical evaluation and electrodiagnostic tests are the only two ways to evaluate nerve outgrowth after repair. Clinical evaluation is a waiting game because it is subject to the rule of axonal growth at a speed of 1 mm/day, and subsequent reinnervation of the muscle.⁸ The relevant information about regeneration will be delayed until the axons reach the end plate. Tinel sign is an important component of sensory level evaluation, which in some patients could be an unpleasant experience, especially if they have neuromas or post-injury pain sequela.^{3,9} Another option, performing an electromyography, also has the disadvantage of being painful because of its invasive nature to access the target muscle. Electromyography is also time sensitive because it cannot be performed before 3 weeks post-injury when denervated fibrillations appear.^{9,10} After repair, axonal regeneration is monitored every 90 days.⁹ But, unless the axonal outgrowth reinnervates the end plate, no relevant information can be obtained about the status of the repair. Unfortunately, this forces us, as health care providers, to adapt a “watch and wait” approach to assess nerve recovery. In some cases, the interval surpasses our expectation allowing irreversible muscular atrophy to set in. Developing a prompt, noninvasive, and accurate tool for nerve outgrowth, assessment has been the focus of our team for the past years.

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) method that measures the effect of tissue barriers on the diffusion of water molecules behavior at a microstructural level. In ordered tissues such as nerves, the effect of tissue barriers is larger perpendicular to the axons than parallel to them, resulting in diffusion anisotropy; changes in anisotropy have been correlated with pathological changes following nerve damage and recovery.^{11,12} From DTI, one can estimate a number of scalar indices, including the axial, radial, and mean diffusivities (AD/RD/MD) as well as the fractional anisotropy ($FA = 0-1$, with higher

values representing higher anisotropy). Axial diffusivities represents the fastest diffusion direction along the axis of the nerve. Radial diffusivities represents slower diffusion in the transverse axis of the nerve, which is modulated by myelin and axonal densities.¹³ Mean diffusivities is the average diffusivity across all directions, which is equivalent to the mean apparent diffusion coefficient.¹² In addition to these scalar indices, diffusion tensor tractography (DTT), which reconstructs fiber tracts based upon the estimated fast diffusion direction in neighboring voxels, can be performed from DTI data.

Scalar DTI indices provide little information within the first 4 weeks of Wallerian degeneration because of the effect of edema. Here, we develop a DTT that included additional orientation information to assess early nerve regeneration 1 week after partial transection and surgical repair. Additional behavioral assessments (sciatic function index [SFI], foot fault [FF] asymmetry) and histology (Toluidine blue staining) were performed for validation purposes.

METHODS

Animals

After obtaining Institutional Animal Care and Use Committee approval (application number M16000-99), as well as Animal Care and Use Review Office approval, female Sprague-Dawley rats between 8 and 12 weeks of age were randomly divided into groups of six animals. The groups included 25, 50, or 75% partial nerve transection as well as sham. All animals were housed in our central animal care facility where they had access to food and water ad libitum with 12 hours light/dark cycle and in accordance with the Guide for Care and Use of Laboratory Animals to minimize pain and suffering.

Surgery

Under general anesthesia with isoflurane 2% at a dose of 3 mL/min, the surgical area was clipped, then prepped with chlorhexidine and draped. All the procedures were performed on the left hind limb. A 3-cm longitudinal incision was performed from the ischial notch down, parallel to the femur's longer axis. Using a split muscle technique, the sciatic nerve was identified and dissected free from the surrounding tissue. The sham group consisted of freeing up the nerve, no intervention and closure. The partial transection injury was created with number 15 stainless steel blade, and the partial injury device shown in [Supplemental Figure 1](#) was placed at least 1 cm proximal to the trifurcation of the nerve. The devices were custom manufactured with varying groove depths to create 25, 50, or 75% injuries using a Monoprice Maker Ultimate 3D Printer, Monoprice, Inc, Rancho Cucamonga, CA and consisted of 100% polylactic acid plastic. Devices were sterilized via ethylene oxide before use in surgery. After transection, the injury was repaired immediately using interrupted epineurial 9-0 nylon sutures (Ethicon, Somerville, NJ) under an operating microscope.

All wounds were irrigated profusely with Ringer's lactated solution and closed in layers using 5-0 Monocryl suture (Ethicon, Somerville, NJ). The animals were monitored in recovery and transported back in stable conditions to our animal facility. All animals received meloxicam (2 mg/kg) subcutaneously daily for 3 days post-procedure for analgesia.

Nerve Samples

Once reaching the first post-operative week, each rat was euthanized under heavy anesthesia with euthasol (Virbac AH, Fort Worth, Texas) at 120 mg/kg placed intracardiac and sciatic nerves were harvested. Harvested nerves were fixed in 2% paraformaldehyde/3% glutaraldehyde in phosphate buffer solution for 24 hours and then washed for 7 (7) days in phosphate buffered solution to remove excess fixative and doped with 1 mM gadolinium diethylene triamine pentaacetic acid (Magnevist, Bayer healthCare, Bayer Healthcare Pharmaceuticals, LLC, Berlin, Germany) to reduce MRI relaxation and scan times.

Behavioral Testing

Clinical evaluations consist of two components: SFI and FF. Our assessments were performed pre-operatively, post-operative day 3 and day 7, and immediately before euthanasia. To obtain SFI, rats were trained to climb a beam, and, after performing without hesitation, each hind paw was inked with different colors and recorded on a paper strip applied to the wooden beam. Three consecutive foot prints with each limb were recorded and measured. The operated limb was considered the experimental, and the contralateral one was identified as normal. Each print was examined, and function was quantified with the following: normal print length (NPL), normal toe spread (NTS), normal intermediary toe spread (NIT), experimental print length, experimental toe spread, and experimental intermediary toe spread. Sciatic Functional Index scores were calculated using the formula: $SFI = -38.3[(\text{experimental print length} - NPL)/NPL] + 109.5[(\text{experimental toe spread} - NTS)/NTS] + 13.3[(\text{experimental intermediary toe spread} - NIT)/NIT] - 8.8$.¹⁴ To obtain FF data, each rat was placed in a grid with 2.5 cm² apertures raised 2 cm from the base, and a total of 50 forward steps with each hind limb was recorded. A full fault was recorded when the posterior foot touched the floor, although a partial fault was recorded when the foot passed through the grid but did not touch the underlying floor. The FF score was calculated using the formula: composite FF score = (n partial faults $\times$ 1) + (n full faults $\times$ 2), %FF = (composite FF score/total number of steps) 100%, FF asymmetry score = %FF (normal hind limb) - %FF (surgical hind limb).¹⁴

Imaging

MRI was performed ex vivo using a 7-T, 16-cm bore Varian Direct Drive scanner and 25-mm quadrature Doty Scientific. DTI data were collected using a 3D pulsed

gradient spin-echo sequence with 20 diffusion directions and $b = 2,000$ s/mm² ($\delta/\Delta = 4/12$ ms). Additional parameters included: FOV = $6 \times 6 \times 16$ mm³, NEX (number of excitations) = 2, TE/TR = 22/425 ms, resolution = $125 \times 125 \times 372$ μ m³ and a scan time = 7 hours, 40 minutes. Diffusion tensors scalar metrics (FA, AD/RD/MD) and diffusion directions (visualized via diffusion ellipsoids) were estimated using in-house written code (MATLAB).¹⁵ Fiber tracking was additionally performed for visualization purposes using the MATLAB toolbox ExploreDTI. Quantification of cut depth in a nerve was obtained by measuring the number of pixels within the nerve, whose correspondent diffusion ellipsoid's major axis was oriented in the x - y axis (i.e., not in the primary direction of the nerve fibers, which were oriented along the z -axis) and divided by the total number of pixels in the corresponding slice. These measurements were performed in each slice of the proximal transected and distal region of the nerve providing the percentage of the cut depth. Voxels at the boundaries of the nerve tissue were excluded from this analysis to minimize the impact of partial volume averaging with surrounding tissue.

Histology

Following MRI scanning, harvested nerves were post-fixed with 1% OsO₄ solution, dehydrated through increasing concentrations of ethanol, and then embedded in resin at 60° for 72 hours. Embedded specimens were sectioned at 1- μ m and stained with 1% toluidine blue. Digital images of sections were acquired on an Olympus C-35 AD-4 microscope. The entire cross-section was imaged at 20X, and additional random 40X images (3 each) were captured from representative healthy and injured regions. Using Image-Pro plus 7.0 software, healthy and injured area measurements were quantified from 20X images. Axons within each 40X image were counted manually using the count tool in Photoshop CS5 Extended Edition, and density (number/mm²) measurements were calculated for healthy and injured areas. Total axon counts were calculated by multiplying axon density by healthy and injured areas calculated from 20X images and then summing the average total axons for the two regions. Percent injury was calculated by comparing total axon measurements made distal to the injury site against total axon measurements proximal to the injury site, with each nerve serving as its own control. Percent injury was averaged for each experimental group.

Statistics

A single factor analysis of variance was performed to test for significant variations across all groups followed by post-hoc pairwise t -tests across each group. After Bonferroni correction, statistical significance was deemed for p -values < 0.05 .

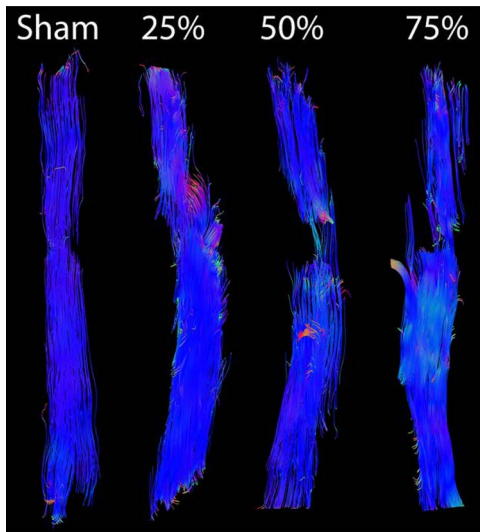


FIGURE 1. DTT of the sham and partially injured rat sciatic nerve. Notice that the sham image has full tracts without interruption. The degree of interruption increases as the partial injury increased.

RESULTS

Fiber tracts for sham and several degrees of partial cuts (25, 50, and 75%) are shown in Figure 1. DTT in sham nerves indicate that all fibers remained intact throughout the length of the nerve. Partially injured nerves show the severity in the discontinuity of fiber tracks in the transected region. Depending on the depth of the injury, the interruption is bigger or smaller in the tractography images. Ellipsoidal maps in a sagittal plane, shown in Supplemental Figure 2, display noninjured regions as blue ellipsoids whose semimajor axis is oriented in the z-direction. Meanwhile, red and green ellipsoids represent orientation in x- and y-directions, indicating disruptions of fiber coherence. In Supplemental Figure 2, the center image shows ellipsoidal maps from multiple axial locations along the nerve, although the right-hand side of the image displays more detailed ellipsoidal maps from the proximal, transected, and distal regions. It is clear that fiber orientation disruption only happens at the transected region, while fibers are more organized in the healthy areas proximal and distal to the injury. When analyzing the ellipsoid's major axis orientations and the fiber disruption at the site injury, Figure 2 illustrates the directionality of the fibers for nerves as grouped by injury depth. Mean values are calculated and represented by black lines. Quantitative assessment of cut severity in Figure 2 indicates that the proposed method can be assay damage severity 1 week after injury/repair.

Upon clinical evaluations of these animals, we see that FF values in all groups decrease on postoperative day 3 consistently compared with baseline (Supplemental Figure 3A). However, the 25% group recovers to almost preoperative values within 1 week. The remaining two groups have a proportional decrease with expected recovery at week 1, in which the 50% cohort was lower than the 25% cohort, but

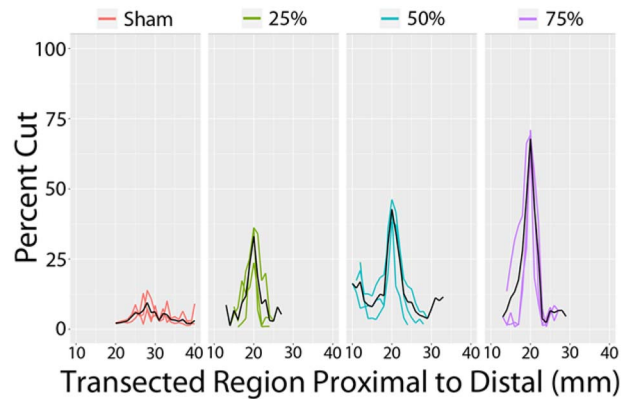


FIGURE 2. Quantitative measurements of transected nerves severity. Sham nerves (0% injury) is used as base line for cut depths of 25, 50, and 75%. Means ($n = 3$) are represented by the black lines.

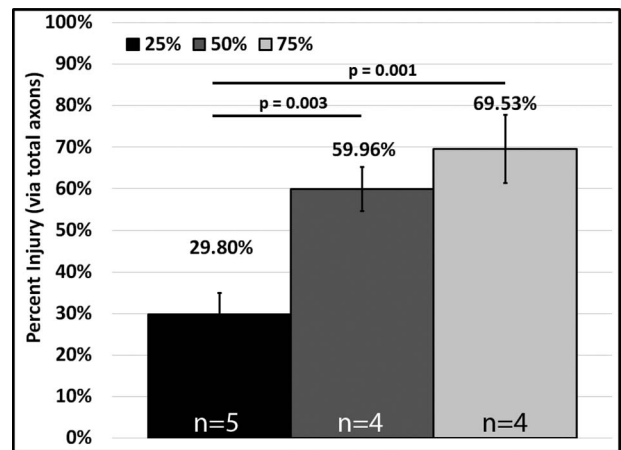


FIGURE 3. Axon counting of the injury area compared with total cross-section of the nerve. Statistical significant difference between 25% group versus 50% group ($p < 0.05$) and 25% group versus 75% group ($p < 0.005$).

higher than the 75% cohort. Regarding SFI, all groups had expected decreased scores on the earliest post-operative evaluation, but recovery was much slower than FF at 1 week (Supplemental Figure 3B). There was virtually no difference between post-operative day 3 values and those at 1 week, except for in the 75% injured, which improved slightly, though still did not approach baseline. Histology methods were also deployed for data for validation, and total axons were calculated from cross-sectional areas proximal and distal to the injury and repair (Fig. 3). The mean area of injury calculated for the 25% group was 29.80% ($\pm 5.21\%$). For the 50% group, the injured area calculated was 59.96% ($\pm 5.33\%$) with statistical significance compared with the former group ($p < 0.005$). Concerning the 75% group, the injured area calculated was 69.53% ($\pm 6.86\%$), also with statistical significance compared with the 25% group ($p < 0.005$).

DISCUSSION

Soldiers injured in action who suffer a peripheral nerve damage are frequently injured by improvised explosive devices.¹⁶ Explosion injuries in periods of conflict have been strongly associated with nerve disability when compared with gunshot wounds, especially proximal to the elbow.⁵ Blast wounds are a result of an extensive amount of energy transferred to the tissues involved. Acute imaging of combat wounds via DTI is not accurate because of the degree of inflammation and edema, which increases isotropy and results in artificial changes in diffusivities that do not reflect injury severity. In this study, we demonstrated that DTI tractography and ellipsoid mapping can overcome this challenge and can provide baseline nerve status following acute injury.

By applying voxel fiber orientation, we assessed the direction of movement of water molecules in the partial injured nerves, obtaining a better idea of these subtle changes in the axonal behavior. The dark blue areas in Supplemental Figure 2 represent fiber orientation in *z*-axis correlated with healthy axonal behavior. These areas are seen proximally and distally to the injury, meaning that there is a large portion of the nerve caudally that is not harmed equally as proximal to the laceration. In other areas represented with polychromatic tones, values around the injury could correlate with isotropic movement of water molecules, meaning fiber orientation is dominant in the *x*- and *y*-axes. By quantifying the injured region and comparing it with the full axonal area, we were able to quantify the injury depth.

For SFI measurements, a score of less than -100 translates to gait impairment, whereas closer to zero is considered normal behavior.¹⁷ Poor scoring is determined by the fact that the distal toe spread is affected compared with the contralateral hind limb. This is controlled by the most distal muscle group of the extremity, requiring the most time to become reinnervated.¹⁷ Previous animal models have been exhaustively studied on how motor dysfunction takes effect with a decrease in axonal population of more than 80%.^{6,7,18} Clinical evaluations of our animals demonstrated that the degree of injury was comparable with their performance. In our study, we noticed that the worst clinical performance was correlated with the 75% injury at postoperative day 3 and with some recovery in the first week after surgery. This phenomenon was more noticeable with FF than in the SFI. Ganguly et al.¹⁷ showed in their study how Sprague-Dawley rats treated with crush and with complete transection performed poorly at postoperative days 3 and 7, in which the crush group only started to recover only after almost day 10 after the intervention. Our rats recovered quicker because of the fact that only a portion of the nerve was affected, and the remaining untouched axons compensated, preventing poor scoring. We also noticed worse clinical performance in the first evaluation of the remaining groups, but in the 25% group, this decline was very subtle and returning to near baseline in

the subsequent evaluation of FF as well. In the half-injured cohort, the performance was in between the extreme groups, correlating with the degree of injury. Although previous work has shown associations between DTI parameters and clinical evaluations like leg contraction, rotarod treadmill (motor), and Von Frey filament, these previous experimental findings were after follow-ups of more than 1 week.¹⁹

Furthermore, when validating our DTT data with behavioral testing, these findings correlate accordingly with the amount of axonal damage. Even though our comparison is qualitative, we were able to pair the degree of injury with gait performance. Previous evaluation of early time points by quantitative DTI has been challenged, prompting the development of new tools, such as ellipsoid mapping to distinguish the varying degrees of injury. We also had the virtue to validate the accuracy of our novel device that we designed to reproduce the percentage of neurite injury. This prototype allows us to reproduce a partial nerve injury in an easy and consistent manner.

Previous animal studies have shown correlation between poor SFI scores and lower axon counts.²⁰ When obtaining histological parameters, total axon counts were performed. The amount of viable myelinated axons correlated linearly with the degree of injury produced to the nerve, in other words, the depth of the groove of the device manufactured in tridimensional plastic printing was associated with the same amount of healthy axonal population. Kakegawa et al.²⁰ attributed the correlation between injury and axonal counts was mostly because of the size of myelinated axons, or possibly because small regenerating axons escape the resolution of light microscopy.²⁰ However, the number of distal axons does not always reflect the amount of cell bodies proximal to the injury possibly because of the axonal sprouting phenomenon. It has been demonstrated that motor axons can sprout into three new axons and sensory can sprout into six.^{6,21} Perhaps performing retrograde labeling could determine the amount of cell bodies located proximal to the injury can be associated to the number of axons caudally to the injury.

The strength of this work is that our proposed technology can detect subtle changes in nerve architecture as early as 1 week. However, this is a murine model that will need to be established in larger animals, before translating to a human. In the past, Boyer et al.²² attempted to study partial injuries of sciatic injuries with DTI. Unfortunately, they were unable to distinguish the percentage of laceration based on FA alone at that time and were unable to validate their results with histology.²² Our group, in agreement with Takagi et al.¹⁹, observed that FA, RD, AD, and MD parameters are obscured by edema. For this reason, we focused our studies using semiquantitative tractography and diffusion directionality.

One of the limitations of this technology is that most soldiers have been injured by explosions that can have shrapnel making DTI impossible. However, with serial débridements,

these fragments could be excised before scanning, though this should be kept in consideration when applying this technology to monitor our wounded soldiers. Nevertheless, obtaining data as early as possible would be extremely beneficial to surgeons, in that they could predict which nerve repairs are going to succeed or fail and thereby determine if another intervention is required, defeating the lengthy race against muscle atrophy. In this study, our results were as early as 7 days, but considering that this was a controlled animal population with an isolated injury, when it comes to translate to humans; we still need to do more studies with complex wounds to be able to apply this technique to our wounded soldiers. The fact that we were able to validate our results this early makes us very optimistic in DTI as peripheral nerve injury monitoring device. The main strength of this technology is not in the acute setting, we believe that the benefits of this monitoring tool is more in the subacute phase, when all life-threatening injuries have subsided, and the remaining question is if nerve function will favor limb salvage or amputation. This was the main reason why we chose to do an isolated nerve injury, to avoid surrounding tissue bias when collecting our data. Using DTT to monitor peripheral nerve outgrowth makes sense because of that they represent fiber tracts that reflect axonal architecture.²³ DTT has proven that this representation can orientate surgeons to preoperatively design a surgical plan when resecting a benign or malignant tumor.²³ The main focus of our laboratory is to find an alternative that can help reintroduce our disabled warriors back in to a productive society or at least routine daily activities. Having premature information about the neurological status of the limb will aid in determining if a surgical intervention (redo neuroorrhaphy, conduit, or nerve transfer) is required to avoid irreversible muscular atrophy. Our results in rat sciatic nerves hold promise for the possible translation of these technologies into humans in vivo.¹⁹

Future studies include an in vivo protocol design allowing us to follow longitudinally, a single larger animal.¹¹ This will allow us to monitor the effects of inflammation and edema in real time and reduce the number of animals studied while minimize suffering. This will also eliminate the detrimental effect that fixation of the nerve has over isotropic diffusion.²⁴ Scanning the same subject will need a qualified team able to induce anesthesia and monitor vital signs, as well as a developed facility. This allocation must be conditioned to perform scans for long periods of time, minimizing motion that could distort the image captured.^{11,23} Additionally, more advanced diffusion models that account for the presence of edema are being investigated.

CONCLUSIONS

These results indicate that orientation information from high-resolution DTT of ex vivo may provide viable biomarkers of peripheral recovery in the presence of edema in a rat sciatic nerve injury model, even in cases where scalar indices are corrupted. These findings suggest that we can differentiate

injury severity via DTI ellipsoids without the necessity to wait for the edema to subside.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *MILMED* online.

REFERENCES

1. Chambers JA, Hiles CL, Keene BP: Brachial plexus injury management in military casualties: who, what, when, why, and how. *J Mil Med* 2014; 179(6): 640–4.
2. Mathieu L, Bertani A, Gaillard C, et al: Surgical management of combat-related upper extremity injuries. *Chir Main* 2014; 33(3): 174–82.
3. Birch R, Misra P, Stewart MP, et al: Nerve injuries sustained during warfare part I—Epidemiology. *J Bone Joint Surg Br* 2012; 94(4): 523–8.
4. Cross JD, Ficke JR, Hsu JR, Masini BD, Wenke JC: Battlefield orthopaedic injuries cause the majority of long-term disabilities. *J Am Acad Orthop Surg* 2011; 19(Suppl 1): S1–7.
5. Rivera JC, Glebus GP, Cho MS: Disability following combat-sustained nerve injury of the upper limb. *Bone Joint J* 2014; 96-B(2): 254–8.
6. Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T: Outcome measures of peripheral nerve regeneration. *Ann Anat* 2011; 193(4): 321–33.
7. Poppler LH, Schellhardt LM, Hunter DA, et al: Selective nerve root transection in the rat produces permanent, partial nerve injury models with variable levels of functional deficit. *Plast Reconstr Surg* 2017; 139(1): 94–103.
8. Moore AM, Wagner II, Fox IK: Principles of nerve repair in complex wounds of the upper extremity. *Semin Plast Surg* 2015; 29(1): 40–7.
9. Bassilios Habre S, Bond G, Jing XL, Kostopoulos E, Wallace RD, Konofaos P: The surgical management of nerve gaps: present and future. *Ann Plast Surg* 2018; 80(3): 252–61.
10. Gilliat RW: Electrophysiology and electromyography in clinical practice. *Br Med J* 1962; 2(5312): 1073–9.
11. Andersson G, Orådd G, Sultan F, Novikov LN: In vivo diffusion tensor imaging, diffusion kurtosis imaging, and Tractography of a sciatic nerve injury model in rat at 9.4T. *Sci Rep* 2018; 8(1): .
12. Jeon T, Fung MM, Koch KM, Tan ET, Sneag DB: Peripheral nerve diffusion tensor imaging: overview, pitfalls, and future directions. *J Magn Reson Imaging* 2018; 47(5): 1171–89.
13. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH: Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage* 2003; 20(3): 1714–22.
14. Nguyen L, Afshari A, Kelm ND, et al: Bridging the gap: engineered porcine-derived urinary bladder matrix conduits as a novel scaffold for peripheral nerve regeneration. *Ann Plast Surg* 2017; 78(6S Suppl 5): S328–34.
15. Veraart J, Sijbers J, Sunaert S, Leemans A, Jeurissen B: Weighted linear least squares estimation of diffusion MRI parameters: strengths, limitations, and pitfalls. *Neuroimage* 2013; 81: 335–46.
16. Ramasamy A, Masouros SD, Newell N, et al: In-vehicle extremity injuries from improvised explosive devices: current and future foci. *Philos Trans R Soc Lond B Biol Sci* 2011; 366(1562): 160–70.
17. Ganguly A, McEwen C, Troy EL, et al: Recovery of sensorimotor function following sciatic nerve injury across multiple rat strains. *J Neurosci Methods* 2017; 275: 25–32.
18. Isaacs J, Mallu S, Wo Y, Shah S: A rodent model of partial muscle re-innervation. *J Neurosci Methods* 2013; 219(1): 183–7.

19. Takagi T, Nakamura M, Yamada M, et al: Visualization of peripheral nerve degeneration and regeneration: monitoring with diffusion tensor tractography. *Neuroimage* 2009; 44(3): 884–92.
 20. Kakegawa A, Yokouchi K, Itsubo T, et al: Correlation between motor function and axonal morphology in neonatally sciatic nerve-injured rats. *Anat Sci Int* 2015; 90(2): 97–103.
 21. Gordon T, Borschel GH: The use of the rat as a model for studying peripheral nerve regeneration and sprouting after complete and partial nerve injuries. *Exp Neurol* 2017; 287(Pt 3): 331–47.
 22. Boyer RB, Kelm ND, Riley DC, et al: 4.7-T diffusion tensor imaging of acute traumatic peripheral nerve injury. *Neurosurg Focus* 2015;39(3):E9.
 23. Vargas MI, Viallon M, Nguyen D, Delavelle J, Becker M: Diffusion tensor imaging (DTI) and tractography of the brachial plexus: feasibility and initial experience in neoplastic conditions. *Neuroradiology* 2010; 52(3): 237–45.
 24. Miller KL, Stagg CJ, Douaud G, et al: Diffusion imaging of whole, post-mortem human brains on a clinical MRI scanner. *Neuroimage* 2011; 57(1): 167–81.
-

Diffusion Magnetic Resonance Imaging Predicts Peripheral Nerve Recovery in a Rat Sciatic Nerve Injury Model

Angel F. Farinas, M.D.
Isaac V. Manzanera Esteve,
Ph.D.

Alonda C. Pollins, M.L.I.
Nancy L. Cardwell, B.S.
Christodoulos Kaoutzanis,
M.D.

Marlieke E. Nussenbaum,
M.D.

Mark D. Does, Ph.D.
Richard D. Dortch, Ph.D.

Galen Perdakis, M.D.
Wesley P. Thayer, M.D.,
Ph.D.

Nashville, Tenn.



Background: Nerve regeneration after an injury should occur in a timely fashion for function to be restored. Current methods cannot monitor regeneration prior to muscle reinnervation. Diffusion tensor imaging has been previously shown to provide quantitative indices after nerve recovery. The goal of this study was to validate the use of this technology following nerve injury via a series of rat sciatic nerve injury/repair studies.

Methods: Sprague-Dawley rats were prospectively divided by procedure (sham, crush, or cut/repair) and time points (1, 2, 4, and 12 weeks after surgery). At the appropriate time point, each animal was euthanized and the sciatic nerve was harvested and fixed. Data were obtained using a 7-Tesla magnetic resonance imaging system. For validation, findings were compared to behavioral testing (foot fault asymmetry and sciatic function index) and cross-sectional axonal counting of toluidine blue-stained sections examined under light microscopy.

Results: Sixty-three rats were divided into three treatment groups (sham, $n = 21$; crush, $n = 23$; and cut/repair, $n = 19$). Fractional anisotropy was able to differentiate between recovery following sham, crush, and cut/repair injuries as early as 2 weeks ($p < 0.05$), with more accurate differentiation thereafter. More importantly, the difference in anisotropy between distal and proximal regions recognized animals with successful and failed recoveries according to behavioral analysis, especially at 12 weeks. In addition, diffusion tensor imaging-based tractography provided a visual representation of nerve continuity in all treatment groups.

Conclusions: Diffuse tensor imaging is an objective and noninvasive tool for monitoring nerve regeneration. Its use could facilitate earlier detection of failed repairs to potentially help improve outcomes. (*Plast. Reconstr. Surg.* 145: 949, 2020.)

In the United States, the prevalence of traumatic peripheral nerve injury in level I trauma centers is about 2.8 percent.¹ Injury can result from penetrating injuries, lacerations, stretch, ischemia, and/or crush mechanisms.² Primary repair after traumatic peripheral nerve injury is

recommended when the severed nerve endings can be approximated without tension, representing approximately half of all these injuries.^{3–5} Regardless of the quality of nerve repair, regeneration is a protracted process that occurs at a rate of 1 mm/day, taking months to reinnervate target muscles.⁶ Previous studies have claimed that reinnervation has to be completed before 16 months after injury to avoid irreversible muscular atrophy.^{7,8} In addition, not all interventions are successful on the first attempt, and a secondary repair may be required.⁹

Currently, the gold standard tests to assess nerve repairs for extremities are electromyography and

From the Departments of Plastic Surgery and Radiology and Radiological Sciences and the Institute of Imaging Science, Vanderbilt University Medical Center; and the Department of Biomedical Engineering, Vanderbilt University. Received for publication July 11, 2018; accepted August 8, 2019.

The first two authors should be considered co-first authors. Presented at the 2018 American Society for Peripheral Nerve Annual Meeting, in Phoenix, Arizona, January 12 through 14, 2018.

Copyright © 2020 by the American Society of Plastic Surgeons

DOI: 10.1097/PRS.0000000000006638

Disclosure: None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article.

nerve conduction studies.¹⁰ In addition to being invasive and uncomfortable for patients, perhaps the most important limitation of these two modalities is that they can only detect valuable motor information when the nerve axons reach their muscular end plate. This has critical implications in patient recovery, since it obligates physicians to adopt a “watch and wait” approach, leading to significant delays in verifying the diagnosis that can result in irreversible atrophy. Consequently, a technique that allows early accurate evaluation of the nerve recovery, which would help to make a timely decision to salvage the repair, could lead to improved outcomes by allowing earlier revision coaptation, nerve grafting, and/or nerve transfers.

Diffusion tensor imaging is a magnetic resonance modality that provides information about tissues by measuring the random movement of water molecules at a microstructural level.¹¹ Water diffusion is isotropic (i.e., the mean-squared displacement of water molecules is the same in all directions) when there are no constrained boundaries.^{7,12} When water is constrained by tissue structures (e.g., packed cylindrical axons), the apparent diffusivity becomes direction dependent or anisotropic. Peripheral nerve studies indicated that apparent diffusivity is lower perpendicular to axons than parallel to them due to the arrangement of nerve fibers.^{7,8,12} Previous authors have shown that fractional anisotropy may be a viable biomarker of nerve degeneration and regeneration.^{13–18} Although previous diffusion tensor imaging studies have predicted the severity of nerve injury, no studies to date have quantified the ability of this technology to stratify successful or failed surgical interventions.¹⁶ In this study, high-resolution ex vivo images of rat sciatic nerves following different injuries at different time points were validated to behavioral and histological data to demonstrate the technique’s ability to follow nerve recover after repair. Interestingly, the technique can detect distal nerve injury and lack of reinnervation at early time points, which might help surgeons determine which nerve repairs will require reinnervation at an earlier time point.

METHODS

Experimental Design

All procedures were reviewed and approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee, which adheres to the *Guide for Care and Use of Laboratory*

Animals to minimize pain and suffering. Sprague-Dawley female rats, between the ages of 8 to 12 weeks, were prospectively divided into groups according to their surgical intervention. Animals were euthanized at different time points after each intervention (1, 2, 4, and 12 weeks), at which point ex vivo scanning was performed. Behavioral tests with foot fault asymmetry score and the sciatic function index were performed before each surgical intervention, on postoperative day 3, and weekly thereafter until each animal was euthanized.

Sciatic Nerve Injury

Isoflurane 2% at a dose of 3 ml/min was used for induction and maintenance of general anesthesia. Hypothermia was avoided during the procedure. A single surgeon (M.E.N.) performed all the procedures. The surgical area was prepared, and a 3-cm longitudinal incision was made from the ischial notch down distally, parallel to the femur. Using a split muscle technique of biceps femoris, the sciatic nerve was identified. The muscle was transected, and the nerve was freed proximally and distally up to its trifurcation. At this point, each nerve received sham, crush, or cut/repair treatment. The sham treatment consisted on no further intervention. An area 1 cm proximal from the trifurcation was selected. For the crush group, a hemostat was applied to the nerve for 10 seconds. For the cut/repair group, the nerve was completely transected and immediately repaired in an end-to-end fashion with interrupted epineurial 9-0 nylon sutures (Ethicon, Somerville, N.J.). All wounds were irrigated thoroughly and closed in two layers using 5-0 Monocryl sutures (Ethicon). The animals were allowed to recover. Upon reaching their time points, each rat was euthanized with an intracardiac dose of Euthasol (Virbac AH, Fort Worth, Texas) at a dose of 120 mg/kg, and the nerves were harvested for imaging.

Tissue Sample Preparation

All nerve samples were washed for at least 1 week in phosphate-buffered saline to remove excess fixation and then immersed in 1 mM gadolinium diethylene triamine penta-acetic acid (Magnevist; Bayer HealthCare LLC, Whippany, N.J.) at 4°C for 36 hours before imaging to reduce spin-lattice relaxation (and corresponding scan) times. Before imaging, all samples were trimmed to 1 cm in length, with crush and cut regions at the center of each nerve segment. The samples were placed in 1.75-mm glass capillary tubes filled with Fomblin (Solvay, Baltimore, Md.) to prevent

dehydration without disrupting the magnetic resonance signal. Six nerves were imaged simultaneously in a hexagonal arrangement.

Diffusion Tensor Imaging Acquisition

Data were acquired in a 7-Tesla, 16-cm bore BioSpec console (Bruker, Rheinstetten, Germany) using a 25-mm quadrature coil (Doty Scientific, Inc., Columbia, S.C.) for transmission and reception. High-resolution scans were performed using an echo time/repetition time of 22/425 msec and field of view of $60 \times 60 \times 160 \text{ mm}^3$, for a nominal resolution of $125 \times 125 \times 372 \text{ }\mu\text{m}^3$. Diffusion tensor imaging was performed using a three-dimensional, diffusion-weighted spin-echo sequence, with a δ/Δ of 4/12 msec, two averages, 20 directions with b of 2000 seconds/ mm^2 (and one b of 0 image), and a scan time of 7 hours 40 minutes.

Diffusion Tensor Imaging Analysis

Analysis was performed on a voxel-wise basis using a linear regression approach written in MATLAB (Mathworks, Inc., Natick, Mass.).¹⁹ Fractional anisotropy was estimated from this analysis. Each slice was classified relative to the injury site (proximal to injury, within zone of injury, distal to injury), and a region of interest was drawn manually at each slice to measure the mean fractional anisotropy along each sciatic nerve. In addition, fiber tracts were reconstructed using the ExploreDTI Toolbox in MATLAB with a fractional anisotropy threshold of 0.4.²⁰ Recovery of fractional anisotropy in the distal region with respect to the proximal region (delta fractional anisotropy) was obtained by subtracting each individual fractional anisotropy value of the distal region from the mean fractional anisotropy in the proximal region of the same nerve. This analysis aimed to eliminate possible inconsistencies across samples. Also, normalization of each nerve's distal fractional anisotropy value to its proximal value provides a quantifiable measurement of nerve recovery.

Behavioral Testing

We obtained measures of foot fault asymmetry score and sciatic function index, as described in previous studies.²¹ An index greater than -10 was considered as recovered, between -30 and -10 was considered partially recovered, and less than -30 was considered not recovered.

Histologic Analysis

Following magnetic resonance imaging, samples were postfixed in 2% paraformaldehyde/3%

glutaraldehyde, counterstained with 1% OsO₄ solution, dehydrated in increasing concentrations of ethanol, and then embedded in resin. Samples were sectioned at 1 μm and stained with 1% toluidine blue for examination with light microscopy (Vanox-T AH-2; Olympus, Tokyo, Japan).

Total axonal cross-sectional areas and axon counts were measured using Image Pro Plus 7.0 (Media Cybernetics, Bethesda, Md.). Within each segment of each nerve, axon density was calculated in triplicate by a single examiner blinded to the experimental group by manually counting axons within three randomly selected areas at 40 $\times$ magnification. Total number of axons was calculated by multiplying each axon density by the nerve cross-sectional area measurement, and then averaging all three to avoid selection bias. Total axon counts were normalized by dividing distal measurements by their corresponding proximal measurements, except for the sham group. In the sham group, the ratio was obtained by dividing the individual measurement by the average of all sham measurements within each time point. Mean differences in axon counts across injury types were compared.

Statistical Analysis

Using R version 3.3.2 software (The R Foundation for Statistical Computing, Vienna, Austria), the Mann-Whitney U test was performed to evaluate differences in sham, crush, and cut/repair injury types. A p value less than 0.05 was considered significant.

RESULTS

Sixty-three Sprague-Dawley rats underwent surgical intervention. They were prospectively divided into three groups: sham surgery, crush injury, or complete transection with primary repair. The unbalanced number of our sample groups was the result of technical difficulties with prolonged fixative exposure, which resulted in some nerves becoming unfit for scanning. The three groups were compared via ex vivo diffusion tensor imaging performed in nerve samples at 1, 2, 4, and 12 weeks postoperatively. [See **Figure, Supplemental Digital Content 1**, which shows the median fractional anisotropy (FA) of procedure groups at all time points. At week 1, all three groups had the same values of fractional anisotropy. As time progressed, there was an increase in fractional anisotropy in all groups, which was more noticeable in the sham and crush groups at as early as 2 weeks. At weeks 2 and 4, the sham group was higher than the crush group ($p < 0.05$; ##, \$\$\$). The cut/repair group differed from both

the sham and crush groups at all time points ($p < 0.05$; *, #, \$, ^) and was not able to reach baseline values at 12 weeks, <http://links.lww.com/PRS/D993>.] In comparison to the crush and cut/repair groups, the sham group displayed the fastest increase in fraction anisotropy, reaching recovery and a plateau by week 4. As expected, the crush group exhibited the second fastest nerve recovery with a path very similar to that of the sham group, but full recovery was not achieved until week 12. Of note, at 1 week postoperatively, the distal fractional anisotropy between the sham, crush, and cut/repair groups showed no statistically significant differences. In contrast, cut/repared nerves were the slowest, with almost no growth up to week 4. At 12 weeks, the distal mean fractional anisotropy values for cut/repair were approximately half those of the sham and crush groups.

Verification of diffusion tensor imaging metrics as a viable biomarker of nerve regeneration was attempted by correlating its results with behavioral studies for each nerve injury, as shown in Figure 1. At week 12, there was a clear correlation between change in fractional anisotropy and behavioral assessments, as recovered and partially

recovered nerves, expressed the lowest change in fractional anisotropy. Nonrecovered nerves expressed the highest values for change in fractional anisotropy and were only present in the cut/repair population at 12 weeks. Thus, a clear contrast in fractional anisotropy values was noted between recovered and nonrecovered nerves in this time period.

Maps of reconstructed fiber tracts with fractional anisotropy overlays, also known as tractography, of the sciatic nerves for each time point and intervention are depicted in Figure 2. In the sham nerves, continuous fiber tracts were noted, the fractional anisotropy values were homogeneously distributed along the nerves, and there was a gradual increase in fractional anisotropy with time until it reached full recovery at 4 weeks. Fiber tracts of crushed nerves showed three different behaviors. First, a large decrease in fractional anisotropy was observed at the injury site that was noticeable at weeks 1, 2, and 4 but totally disappeared by week 12. Second, the distal region fractional anisotropy values demonstrated a slight decrease at week 1, with a gradually recovery through weeks 2 and 4 and reaching full recovery at week 12. It was also

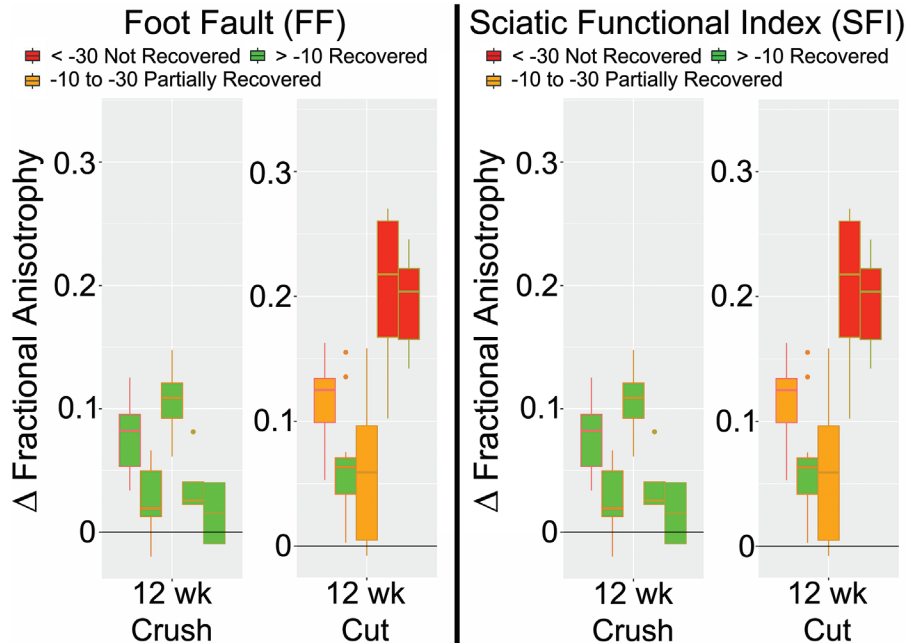


Fig. 1. The differential between proximal and distal fractional anisotropy (ΔFA) of crushed nerves and cut/repared nerves compared with foot fault asymmetry and sciatic function index at different time points. All nerves assessed as recovered (greater than -10) or partially recovered (greater than -30) by the behavioral data are color-coded as green or orange, respectively. At 12 weeks, when regeneration and recovery were possibly achieved, in subjects that did not recover clinically, there was a significant correlation between high ΔFA values and low clinical scores (less than -30 on foot fault and sciatic function index) in the cut/repair group (red).

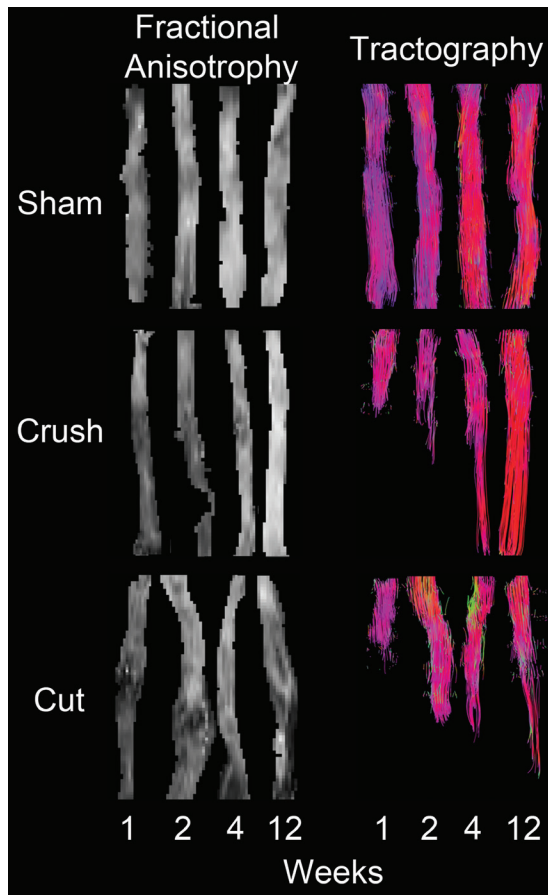


Fig. 2. Representative fractional anisotropy and tractography at each time point by treatment. There was no disruption of tracing throughout the different time points in the sham group. For the crush cohorts, there was progression of tracings throughout the time points that can be translated as recovery. At 12 weeks, there was no difference between the sham and crush tractographs. In the cut/repair group, there was tracing beyond the area corresponding to the coaptation, which correlates with regeneration.

noted that fractional anisotropy intensities in the proximal region were very similar to those of sham nerves at corresponding times. Third, fiber tracts demonstrated a continuous recovery of the fibers over time, with a few fibers completely recovered by week 4 and total recovery by week 12. However, for cut/repared nerves, the coaptation region showed a protuberance at weeks 1 and 2, with a marked decrease in the fractional anisotropy intensity distally. At weeks 4 and 12, the protuberance almost faded entirely; however, fractional anisotropy beyond the repair never reached values of sham or crushed nerves. In addition, at the distal region, the fractional anisotropy was very low at week 1 and recovery was not perceptible until week 12. Finally, tractography images showed that

tracing of the new fibers began at week 4, and it was even more noticeable at week 12.

Histologically, in quantitative analysis (Fig. 3), at weeks 1 and 2 there was a significant difference between sham and crush axon counts in the cut/repair group ($p < 0.05$), while there was no difference between crush and sham axons. By week 4, there was a statistical difference between sham and both crush and cut/repair axons ($p < 0.05$); however, no difference existed between crush and cut/repair axons. Finally, at 12 weeks, there was no difference in any treatments.

DISCUSSION

This prospective animal study has quantified the ability of ex vivo diffusion tensor imaging to stratify successful or failed surgical interventions after nerve injuries. Earlier work by Yamasaki et al.²² examined crush injuries in rabbit sciatic nerves and compared them with the normal contralateral nerves. They compared diffusion tensor imaging results with clinical and histological data and were able to correlate fractional anisotropy with nerve integrity. A decrease in fractional anisotropy in their earlier time points was attributable to myelin sheath disruption. Other studies have reported a decrease in fractional anisotropy in the central nervous system due to chronic degenerative disease and in the peripheral nervous system due to compressive entities or Wallerian degeneration.^{17,23,24} Our analysis demonstrated that inflammation and edema following the injury can affect the assessment of severity with this technique. This is attributed to the abnormal behavior of water in the different cellular compartments of the nerves, resulting in indistinguishable fractional anisotropy values in all three injury types at week 1. However, even in early stages after the injury, tractography can at least differentiate between sham injury and the other injury types (Fig. 2).

Tractography is considered a qualitative three-dimensional axonal representation that can reveal abnormalities beyond magnetic resonance resolution, and it allows early noninvasive nerve regeneration monitoring, better than fractional anisotropy maps.²⁵ At week 2, edema starts to diminish and slight differences among the injury types can be seen with fractional anisotropy. Lehmann et al.¹³ evaluated sciatic nerves in a murine study and showed a higher fractional anisotropy in the control and crush groups when compared with the transected group. In addition, Takagi et al.¹⁷ examined crush sciatic nerve injuries in rats with

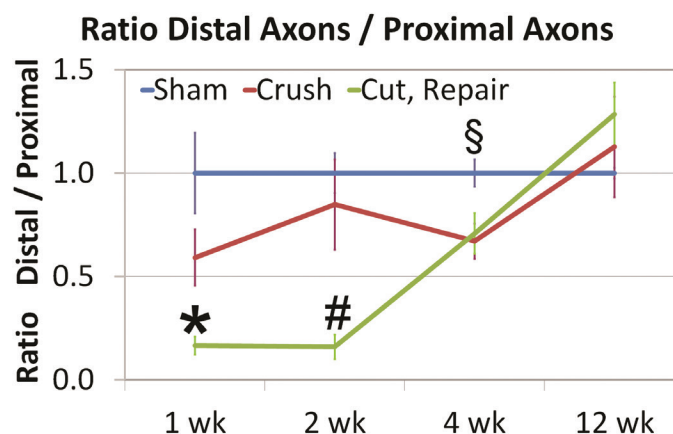


Fig. 3. Distal/proximal total axon ratios for each treatment group at each time point. The sham ratio (*blue*) is 1 because the number of axons distally is the same as the number proximally. At week 1, there were statistical differences between cut/repair (*green*) versus sham and crush (*red*) groups, mostly due to Wallerian degeneration (* $p < 0.05$). At week 2, the significant discrepancies remained (# $p < 0.05$). At 4 weeks, there was an increase in the ratio of the cut/repair group, equal to that of the crush group but below that of the sham group (\$ $p < 0.05$) (sham versus crush and cut/repair). At 12 weeks, the intervention groups were higher than the sham group, due to regeneration and recovery. No statistical significance was observed at this time point. Bar represents standard error. Scale bar, 100 μ m.

fractional anisotropy 5 mm proximal to the injury epicenter, at the epicenter, and 5 mm caudal to the injury. When compared with normal contralateral nerves, the injured nerves showed a drastic drop in fractional anisotropy at the epicenter 4 days after the injury, with recovery to baseline levels at 3 weeks. These results correlated with axon density and behavioral tests (e.g., leg muscle contraction); however, this work was limited to crush injuries only.¹⁷ Our findings suggest that the ideal time to distinguish between different types of nerve injury with diffusion tensor imaging is at 4 weeks, when inflammation has subsided, crushed axonal recovery is mostly completed, and repaired nerves are beginning to regenerate. This may have major implications for nerve injury patients, as this is a potentially powerful noninvasive tool for assessing failure of nerve recovery early after repair. For proximal injuries, this could allow for earlier detection of failed repairs before distal targets atrophy or become nonreceptive for reinnervation. This novel discovery could be animal specific. Future studies must be designed to prove if this finding could be translated across species.

In our study, it was clear from the correlation of the behavioral and imaging data that all crush injuries returned to normal at 12 weeks. Conversely, clinical improvement of cut/repair injuries

was not consistent, with nerves demonstrating a wide spectrum of recovery. After utilizing the differential fractional anisotropy between the regions distal and proximal to the injury, it was possible to correlate imaging with clinical recovery. This single parameter predicted which rats recovered clinically from both injuries at 12 weeks. Performing supplemental tractography also confirmed which nerves regenerated at that time point.

Previous studies advocated that an increase in fractional anisotropy was correlated with an increase in number of axons.¹⁴ Likewise, our data showed an increase in the number of axons when fractional anisotropy increased in all groups, but there were no statistical differences among the three groups at 12 weeks. At this time point, diffusion tensor imaging recognized and classified in a noninvasive fashion the types of injury, regeneration, and even clinical recovery. An increase in axonal population was noted after inflammation subsided and regeneration or recovery was completed. In some cases, the number of axons did not correlate with behavioral improvement at 12 weeks. Riley et al.²⁶ showed no clinical improvement at this same time period, despite an increased axon count in rat sciatic nerve treated with repair plus an axonal fusion agent. This could be explained by the failure to reestablish a successful connection to

the targeted muscle.²⁶ Another theory that could explain this phenomenon is the possibility of axonal sprouting trying to reach the target muscle. In the future, we will try to follow the injured/ repaired axons distally with tractography, in an attempt to decipher this dilemma. Also, we will include other histomorphometric parameters in our subsequent studies, such as myelin thickness and percentage of nerve tissue. Gathering more information at the cellular level could enlighten our knowledge about this situation.

This study has some limitations that merit discussion. First, performing prolonged scans was only possible with rat sciatic nerves after harvest, which eliminated motion artifacts and prolonged use of anesthetics. Ideally, it will be more accurate to monitor the same injured subject through time; we have developed a protocol for this in our ongoing human diffusion tensor imaging/magnetic resonance imaging trials. In addition, diffusion tensor imaging offers more parameters (e.g., axial diffusivity and radial diffusivity) that can also evaluate recovery other than fractional anisotropy. Further investigations are needed to determine the value of these diffusion tensor imaging parameters compared with our findings.

CONCLUSIONS

Our analysis demonstrated that after traumatic nerve injury, high-resolution diffusion tensor imaging measurements could monitor regeneration by identifying injury type and surgical recovery in a rat model. To the best of our knowledge, this is the first study to date to correlate imaging findings with both behavioral and histologic evaluations in crush and cut/repair models. In addition, by calculating the recovery of fractional anisotropy in the distal nerve region with respect to the proximal region, we were able to eliminate inconsistencies among samples, minimize the diffuse effect of edema, and more accurately quantify nerve recovery. The diffusion tensor imaging/magnetic resonance imaging technology has great potential to efficiently predict noninvasively which nerve injuries might require an intervention. This is critically important for patients with failed repair of a proximal nerve injury, in which an early intervention could avoid muscular atrophy.

Wesley P. Thayer, M.D., Ph.D.
Department of Plastic Surgery
Vanderbilt University Medical Center
1161 21st Avenue S, MCN D4207
Nashville, Tenn. 37232-2345
wesley.thayer@vanderbilt.edu

ACKNOWLEDGMENTS

This work was supported by U.S. Army Medical Research and Materiel Command, contract no. W81XWH-15-JPC-8/CRMRP-NMSIRA, grant no. MR150075. Additional support was provided by National Institutes of Health/National Institute of Neurological Disorders and Stroke grant R01 NS97821 (to R.D.D.).

REFERENCES

1. Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma* 1998;45:116–122.
2. Taylor CA, Braza D, Rice JB, Dillingham T. The incidence of peripheral nerve injury in extremity trauma. *Am J Phys Med Rehabil*. 2008;87:381–385.
3. Moore AM, Wagner JJ, Fox IK. Principles of nerve repair in complex wounds of the upper extremity. *Semin Plast Surg*. 2015;29:40–47.
4. Castillo-Galván ML, Martínez-Ruiz FM, de la Garza-Castro O, Elizondo-Omaña RE, Guzmán-López S. [Study of peripheral nerve injury in trauma patients]. *Gac Med Mex*. 2014;150:527–532.
5. Li A, Hokugo A, Yalom A, et al. A bioengineered peripheral nerve construct using aligned peptide amphiphile nanofibers. *Biomaterials* 2014;35:8780–8790.
6. Ovalle F Jr, Patel A, Pollins A, et al. A simple technique for augmentation of axonal ingrowth into chondroitinase-treated acellular nerve grafts using nerve growth factor. *Ann Plast Surg*. 2012;68:518–524.
7. Pasternak O, Sochen N, Gur Y, Intrator N, Assaf Y. Free water elimination and mapping from diffusion MRI. *Magn Reson Med*. 2009;62:717–730.
8. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. *Neurotherapeutics* 2007;4:316–329.
9. Vastamäki M, Kallio PK, Solonen KA. The results of secondary microsurgical repair of ulnar nerve injury. *J Hand Surg Br*. 1993;18:323–326.
10. Han D, Lu J, Xu L, Xu J. Comparison of two electrophysiological methods for the assessment of progress in a rat model of nerve repair. *Int J Clin Exp Med*. 2015;8:2392–2398.
11. Beaulieu C. The basis of anisotropic water diffusion in the nervous system: A technical review. *NMR Biomed*. 2002;15:435–455.
12. Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron* 2006;51:527–539.
13. Lehmann HC, Zhang J, Mori S, Sheikh KA. Diffusion tensor imaging to assess axonal regeneration in peripheral nerves. *Exp Neurol*. 2010;223:238–244.
14. Morisaki S, Kawai Y, Umeda M, et al. In vivo assessment of peripheral nerve regeneration by diffusion tensor imaging. *J Magn Reson Imaging* 2011;33:535–542.
15. Stanis GJ, Midha R, Munro CA, Henkelman RM. MR properties of rat sciatic nerve following trauma. *Magn Reson Med*. 2001;45:415–420.
16. Boyer RB, Kelm ND, Riley DC, et al. 4.7-T diffusion tensor imaging of acute traumatic peripheral nerve injury. *Neurosurg Focus* 2015;39:E9.
17. Takagi T, Nakamura M, Yamada M, et al. Visualization of peripheral nerve degeneration and regeneration: Monitoring with diffusion tensor tractography. *Neuroimage* 2009;44:884–892.

18. McAllister RM, Gilbert SE, Calder JS, Smith PJ. The epidemiology and management of upper limb peripheral nerve injuries in modern practice. *J Hand Surg Br*. 1996;21:4–13.
19. *MATLAB Release 2016*. Natick, Mass: Mathworks, Inc; 2016.
20. Leemans A, Jeurissen B, Sijbers J, Jones DK. ExploreDTI: A graphical toolbox for processing, analyzing and visualizing diffusion MR data. *Proc Intl Soc Mag Reson Med*. 2009;17:3536.
21. Nguyen L, Afshari A, Kelm ND, et al. Bridging the gap: Engineered porcine-derived urinary bladder matrix conduits as a novel scaffold for peripheral nerve regeneration. *Ann Plast Surg*. 2017;78(6S Suppl 5):S328–S334.
22. Yamasaki T, Fujiwara H, Oda R, et al. In vivo evaluation of rabbit sciatic nerve regeneration with diffusion tensor imaging (DTI): Correlations with histology and behavior. *Magn Reson Imaging* 2015;33:95–101.
23. Kimura-Ohba S, Yang Y, Thompson J, et al. Transient increase of fractional anisotropy in reversible vasogenic edema. *J Cereb Blood Flow Metab*. 2016;36:1731–1743.
24. Jeon T, Fung MM, Koch KM, Tan ET, Sneag DB. Peripheral nerve diffusion tensor imaging: Overview, pitfalls, and future directions. *J Magn Reson Imaging* 2018;47:1171–1189.
25. Khalil C, Budzik JF, Kermarrec E, Balbi V, Le Thuc V, Cotten A. Tractography of peripheral nerves and skeletal muscles. *Eur J Radiol*. 2010;76:391–397.
26. Riley DC, Boyer RB, Deister CA, et al. Immediate enhancement of nerve function using a novel axonal fusion device after neurotmesis. *Ann Plast Surg*. 2017;79:590–599.

RESEARCH ARTICLE

Initial findings in traumatic peripheral nerve injury and repair with diffusion tensor imaging

Michael D. Pridmore¹ , Gabriella E. Glassman², Alonda C. Pollins², Isaac V. Manzanera Esteve² , Brian C. Drolet², Douglas R. Weikert³, Mark D. Does⁴, Galen Perdakis², Wesley P. Thayer^{2,a} & Richard D. Dortch^{1,4,5,a}

¹Vanderbilt Institute for Imaging Science, Vanderbilt University Medical Center, Nashville, Tennessee

²Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee

³Department of Orthopaedic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee

⁴Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee

⁵Department of Neuroimaging Research, Barrow Neurological Institute, Phoenix, Arizona

Correspondence

Richard Dortch, 350 W. Thomas Rd.,
Phoenix, AZ 85013-4496. Tel: +1 602-406-
3731; Fax: +1 615-322-0734 ; E-mail:
richard.dortch@barrowneuro.org

Funding Information

This research was supported by grants from the National Institute of Health/National Institute of Neurological Disease and Stroke R01 NS097821 (RDD) and Department of Defense W81XWH-15-JPC-8/CRMRP-NMSIRA, MR150075 (WPT).

Received: 23 July 2020; Revised: 19
November 2020; Accepted: 19 November
2020

doi: 10.1002/acn3.51270

^aThese authors supervised this work equally

Abstract

Objective: Management of peripheral nerve injuries requires physicians to rely on qualitative measures from patient history, electromyography, and physical exam. Determining a successful nerve repair can take months to years for proximal injuries, and the resulting delays in clinical decision-making can lead to a negative impact on patient outcomes. Early identification of a failed nerve repair could prevent permanent muscle atrophy and loss of function. This study aims to test the feasibility of performing diffusion tensor imaging (DTI) to evaluate injury and recovery following repair of wrist trauma. We hypothesize that DTI provides a noninvasive and reliable assessment of regeneration, which may improve clinical decision-making and alter the clinical course of surgical interventions. **Methods:** Clinical and MRI measurements from subjects with traumatic peripheral nerve injury, carpal tunnel syndrome, and healthy control subjects were compared to evaluate the relationship between DTI metrics and injury severity. **Results:** Fractional anisotropy from DTI was sensitive to differences between damaged and healthy nerves, damaged and compressed nerves, and injured and healthy contralateral nerves. Longitudinal measurements in two injury subjects also related to clinical outcomes. Implications of other diffusion measures are also discussed. **Interpretation:** DTI is a sensitive tool for wrist nerve injuries and can be utilized for monitoring nerve recovery. Across three subjects with nerve injuries, this study has shown how DTI can detect abnormalities between injured and healthy nerves, measure recovery, and determine if re-operation was successful. Additional comparisons to carpal tunnel syndrome and healthy nerves show that DTI is sensitive to the degree of impairment.

Introduction

Peripheral nerve damage following injury can result in catastrophic clinical outcomes if not detected and treated in a timely manner. Etiologies of peripheral neuropathy can be divided into traumatic and nontraumatic causes. Traumatic peripheral nerve injury (TPNI) typically has a devastating impact on function and quality of life. TPNI may result in temporary or permanent paralysis, irreversible muscle atrophy, and/or formation of painful

neuromas.¹ TPNI occurs in 3-5% of Level 1 trauma cases and results in approximately 100,000 operative procedures in North America annually,^{2,3} with many additional cases noted in recent military missions.^{4,5} Literature has shown^{1,2,6,7} that TPNI affects the upper extremity more frequently than other areas.

Neurotmesis in the upper extremity is a common, but potentially devastating, injury wherein all components of a nerve (axon, endoneurium, perineurium, and epineurium) are completely transected. This significant injury

warrants surgical repair to re-establish proximal and distal connections. Successful recovery requires axonal regeneration from the repair site to the target tissue at a growth rate of approximately one inch per month.¹ Nerve transfers can reduce the length of axonal growth required to reach target tissue, and nerve grafts provide a conduit to create less tension on the repair; however, approximately 40% of surgical repairs fail following these injuries.^{8,9} Clinical management following a TPNI generally entails ongoing clinical evaluation combined with electrodiagnostic testing. This “watch and wait” methodology takes weeks to months, which can be frustrating for patients and their physicians. Using this management, failure of nerve regeneration often goes unnoticed for extended periods, during which the window of opportunity for a revisional surgery closes, leading to irreversible muscle atrophy due to prolonged muscle denervation.¹⁰

Current diagnostic tools following a traumatic extremity injury are limited to clinical examinations and electrodiagnostic tests.¹¹ In particular, nerve conduction studies (NCS) and electromyography (EMG) remain the mainstay for defining the severity and distribution of motor and sensory function following a TPNI. However, reinnervation typically does not begin until 3–6 weeks following an inciting event. During regeneration, NCS and EMG together provide an incomplete picture of nerve pathology.¹² Additionally, these tests often fail to discriminate neurotmesis or axonotmesis, involving division of axons and elements of the epineurium, from a mild self-resolving neuropraxic injury,^{11–15} such as compression, in the acute setting prior to surgical intervention.

Given the time-sensitive nature of nerve regeneration, high-resolution ultrasonography (HRUS) and magnetic resonance neurography (MRN) imaging have been proposed to mitigate current diagnostic limitations and to explore mapping of peripheral nerve regeneration. HRUS can accurately identify transected nerves,^{16,17} but its use can be limited in traumatic injuries due to associated hematomas, edema, skin lacerations, and alterations of the normal anatomy.¹⁶ Conventional magnetic resonance imaging (MRI) of nerves allows for both longitudinal and cross-sectional fascicular images to detect nerve discontinuity, localize nerve compression, and decipher intraneural from perineural masses.¹⁸ Areas affected by peripheral neuropathy demonstrate hyperintense changes on MRI T₂-weighted sequences that can characterize myelin and axonal health; however, MRN alone cannot discriminate between myelin and axonal pathologies and, therefore, lacks specificity.¹⁹

Diffusion tensor imaging (DTI) is a quantitative MRI method that has been particularly effective in detecting and monitoring central nervous system (CNS) pathologies such as traumatic brain injury, spinal cord injury, and

multiple sclerosis.^{20–22} This method has also shown promising results in the detection of myelin and axon pathology for peripheral nerves like those in the wrist.^{23,24} DTI has previously been used to detect peripheral nerve regeneration as early as 1–3 months in humans.^{25–27} DTI provides a noninvasive, quantitative approach to evaluate tissue microstructures throughout the recovery process by measuring the diffusion of water molecules in tissue over multiple directions.²⁸ In the absence of barriers, diffusion is isotropic or equal in all directions. Conversely in ordered, elongated biological tissue like axons, water diffusion is anisotropic due to its interactions with surrounding tissue structures and cellular membranes, resulting in an apparent diffusivity that is the highest along the primary direction of the axons.^{25,28,29}

Given this sensitivity to nerve microstructure, we aimed to determine the sensitivity of DTI in monitoring nerve degeneration and regeneration following injury and surgical repair. In previous animal studies, we and others demonstrated that DTI could successfully identify and grade complete and partial transections of sciatic nerves in *ex vivo* rats.^{30,31} In this study, we employed DTI on distal nerves of the wrists of human subjects to longitudinally evaluate nerve repair and regeneration. In addition, we included a cohort of carpal tunnel syndrome (CTS) patients to compare the sensitivity of this method to less severe, compression injuries. If successful, these DTI strategies could help improve patient outcomes by predicting regenerative failures earlier than current techniques, and even provide support for early re-operation.

Methods

Human subjects and clinical information

Three subjects with TPNI, eight subjects with CTS, and eight healthy control subjects were consecutively enrolled in this study. All TPNI subjects underwent microsurgical nerve repairs of fully lacerated median and/or ulnar nerve (s) at the Vanderbilt University Medical Center (VUMC) by fellowship-trained hand surgeons. All CTS subjects underwent open carpal tunnel release surgery and were treated with the standard clinical care procedure at VUMC. All healthy controls were volunteers recruited to match the sex and range of ages of the TPNI cohort. Subjects and healthy volunteers were excluded from recruitment if they reported a history of other concurrent neuropathies (e.g., diabetic neuropathies) or wrist trauma. One CTS subject was excluded due to an additional Charcot-Marie-Tooth Type 1A diagnosis, and one TPNI subject was excluded due to a radial nerve injury at the thumb, which resided outside our imaging coil coverage.

For TPNI cases, the nerves were examined using standard microsurgical techniques and the damage was identified as follows: transected nerve endings were inspected, edges were refreshed to healthy bulging fascicles, and an epineural repair was performed with 8-0 nylon sutures, taking care to all align vascular structures to optimize fascicular repair. In carpal tunnel release surgery, an incision was made at the base of the palm radial to the hook of the hamate. Through this incision, surgeons located the transverse carpal ligament (TCL), which lies between the skin and the median nerve in the carpal tunnel. By dividing the TCL, compression of the median nerve was relieved.

As detailed in Table 1, the median nerve was affected by compression in the CTS cohort. In the TPNI cohort, two of the three TPNI subjects had an injury where only one nerve was affected (TPNI 1 and TPNI 3). The other TPNI subject had both the median and the ulnar nerve injured (TPNI 2) on the right arm and was dual-enrolled in another clinical trial at VUMC in which a sealant material composed of polyethylene glycol (PEG) and hypotonic calcium-free saline was applied directly to the repaired nerve adjunctive to the microsurgical repair. PEG has demonstrated safety in human usage³² and PEG fusion has proven effective in terms of functional outcomes and speed of nerve recovery in human digital nerves when compared with standard nerve repair.^{33,34}

More specifically, TPNI 1 presented with a linear laceration of the median nerve in the dominant right arm. Along with repair of the median nerve, surgical repair was done on the complete transections of the flexor carpi radialis tendon, the palmaris longus tendon, the flexor pollicis longus tendon, and partial transection of the flexor distal phalanx tendon. TPNI 2 presented with full transection of the median nerve and partial transection of the ulnar nerve in the dominant right arm. Concomitant with this trauma was a flexor tendon injury, which was also repaired. TPNI 2 received PEG gel as part of the median nerve repair. TPNI 3 presented with partial transection of the ulnar nerve on the nondominant left arm. Along with nerve trauma, TPNI 3 had damaged the flexor carpi ulnaris and flexor digitorum superficialis tendons. Additionally, carpal tunnel release surgery was performed on the median nerve as part of the repair procedure. After clinical information from the second timepoint indicated that the initial repair surgery was not successful, TPNI 3 was re-operated on and a 70 x 5 mm cadaveric nerve graft (Avance) was used to promote repair.

All TPNI subjects underwent an initial MRI one to three months after surgical repair. Additional longitudinal data were collected in three-month intervals following the initial scans in two of the three TPNI subjects. All CTS subjects had postoperative MRI one to five months after carpal tunnel release surgery, with the exception of one

subject who had preoperative MRI less than a month before surgery. Additional longitudinal data were collected in six of the eight CTS subjects when available. Additional clinical assessments were conducted on TPNI subjects, including: Michigan Handedness Questionnaire (MHQ), Grip test, and 9-hole Peg Test (9-HPT). Clinical assessments for CTS subjects are not reported. During the consenting process, all subjects self-reported no symptoms that were suggestive of peripheral nerve disease and physical examination indicated no evidence of any signs of other peripheral neuropathies. Each subject's demographic information and clinical scores (when available) are reported in Table 1.

Standard protocol approvals, registrations, and patient consents

The study was approved by our local Institutional Review Board and all participants provided informed consent prior to all examinations.

Data availability statement

Deidentified data related to this study will be made available from the corresponding author upon request.

MRI data acquisition

Subjects were imaged in the prone position with one arm extended above the head (i.e., "superman" position) with a 3.0-T Philips Ingenia MR scanner. An 8-channel wrist coil was used for RF reception in all subjects except one, where a larger 16-channel knee coil was used due to a cast covering the arm. In each MRI session for TPNI subjects, the arm with the most recent injury and surgery was scanned followed by the contralateral, uninjured arm as an internal control. For CTS subjects, three out of the eight subjects had bilateral CTS, therefore the arm with the most recent surgery was reported in our results to ensure consistency across CTS subjects. Healthy subjects had only one arm scanned, as there was no expectation to have differences between healthy nerves.³⁵ MR images were acquired in both proximal and distal areas from the injury site, covering from mid-forearm to the base of the palm. More specifically, for TPNI subjects imaging was not centered over the region of transection (i.e., the center slice is not the injury site). This was due to limited coil coverage, heterogeneity of injuries, and uncertainty of anatomical landmarks to where injury occurred. Nevertheless, these varying injuries in TPNI subjects were captured within our imaging volume in all subjects. A single-shot EPI scan with diffusion-weighting was performed at slice thicknesses of 4-mm with 10-16 slices in each

Table 1. Demographic information and clinical scores for all subjects. This includes Michigan Handedness Questionnaire (MHQ) and 9-hole Peg Test (9-HPT). Scores of N/A indicate where measures were not obtained. Scores listed as "unable" may also be considered a score of zero. Multiple scores for TPNI 2 and TPNI 3 reflect measures gained at subsequent clinical visits. R = Right arm and L = Left arm. M = Median nerve and U = Ulnar nerve. Time listed in months since surgery

Subject	Demographics:			Time since surgery: (months)	MHQ:		Grip test:		9-HPT:	
	Age (Sex)	BMI	Arm - Nerve		Injured	Healthy	Injured	Healthy	Injured	Healthy
TPNI 1 ²	26 (F)	19.7	R - M	1	N/A	N/A	N/A	N/A	N/A	N/A
TPNI 2	22 (M)	19.8	R - M	1	33	83.3	Unable	92	Unable	23
			& U	4	69	83.3	30	100	100	27
TPNI 3	52 (M)	31.8	L - U	3	5	75.1	30	75	43	27
				6	0.8	81.5	18	74	51	26
				9 ¹	5.8	81.5	N/A	N/A	N/A	N/A
Group:	33.3 (66% M)	31.8								
CTS 1	73 (M)	23.5	R - M	5, 16, 20, 24						
CTS 2	48 (F)	34.4	R - M	4.5, 13.5, 16.5						
CTS 3	58 (F)	26.6	R - M	1, 12, 16						
CTS 4	40 (M)	26.6	R - M	4, 14						
CTS 5	44 (F)	30.8	L - M	<1, 3.5						
CTS 6	48 (F)	38.4	R - M	1, 14						
CTS 7	52 (F)	32	R - M	6						
CTS 8	68 (M)	31.4	R - M	2						
Group:	53.3 (38% M)	30.5								
Control 1	57 (M)	23.1	R - M							
Control 2	54 (F)	32.7	R - M							
Control 3	23 (M)	23.8	R - M							
Control 4	27 (M)	19.3	R - M							
Control 5	27 (M)	28.8	R - M							
Control 6	20 (M)	26.5	R - M							
Control 7	25 (M)	30.1	R - M							
Control 8	26 (M)	21	R - M							
Group:	32.4 (88% M)	25.7								

¹Indicates measures acquired after second surgery in TPNI 3.

²Indicates knee coil was used during MRI acquisition for TPNI 1.

subject. Additional parameters included: resolution = $0.75 \times 0.75 \times 4 \text{ mm}^3$ (wrist coil) or $1.25 \times 1.25 \times 8 \text{ mm}^3$ (knee coil), TR/TE = 3000/53 ms, number of acquisitions = 12, 16 directions, max b-factor of 800 s/mm^2 , and scan time $\approx 11 \text{ min}$.

MRI data analysis

MATLAB (The Mathworks, Inc., Natick, Massachusetts, USA) was used for tensor estimation and image registration. Regions of interest (ROIs) were manually selected using the MIPAV³⁶ imaging software on all slices for the injured and contralateral nerve(s), and mean slice-wise values were estimated for fractional anisotropy (FA = 0–1 with increasing values indicating higher anisotropy), mean diffusivity (MD, mean diffusivity in all directions), radial diffusivity (RD, diffusivity perpendicular to axons), and axial diffusivity (AD, diffusivity parallel to axons). Due to the inherent distortion of EPI data and the lack of robust nonrigid registration tools for upper extremity applications, ROIs were drawn directly on the diffusion data. To minimize bias, these ROIs were drawn on the nondiffusion-weighted (b0) image rather than the parametric maps. The resulting ROI values are reported in Table 2.

Statistical analysis

All statistical analyses were conducted using MATLAB Statistics and Machine Learning Toolbox (The Mathworks, Inc.). The measures for comparison derived from DTI included FA, MD, RD, and AD. Nonparametric statistical approaches were used in this study's analysis and raw p-values are reported given the exploratory nature of this study. Slice-wise variations in FA were evaluated via a Kruskal–Wallis test to see if healthy nerves in TPNI contralateral arms exhibited significant variations along the length of the nerve. Next, we conducted a cross-sectional analysis of all cohorts and timepoints to evaluate the effect of injury severity on the observed DTI metrics in nerves via Wilcoxon Rank-Sum tests. In addition to this, we compared pairwise DTI metrics from injured and contralateral nerves in the same TPNI patients via Wilcoxon Signed-Rank tests. Finally, we compared FA of injured and healthy nerves in individual TPNI subjects longitudinally (when available) via Wilcoxon Rank-Sum tests to assess each subjects' recovery over time.

Results

Clinical data

Clinical measures obtained from all subjects in injured and healthy arms are summarized in Table 1. TPNI 1 did

not participate in these clinical measures. Clinical findings for TPNI 2 show improvement between timepoints (1/2) across all measures for MHQ (33.0/69.0), Grip test (Unable/30), and 9-HPT (Unable/100 sec.). TPNI 3 participated in all clinical assessments for the first and second timepoints, but only participated in the MHQ assessment at the third timepoint. Results of TPNI 3 show a decline in all measures between timepoints (1/2) for MHQ (5.0/0.8), Grip test (30/18), and 9-HPT (43 sec./51 sec.). After re-operation, the MHQ score rose above the first measure (5.8). CTS subjects received standard care, which did not include additional clinical measurements.

Representative data in control, TPNI, and CTS

The MRI scanning procedure was well-tolerated by all subjects. The MR images acquired were artifact-free from chemical shift due to fat and had very little motion artifacts. In the anatomical and diffusion images, the nerves were easily distinguishable from surrounding tissue (Fig. 1).

As expected, there was no effect of slice position on the DTI values from healthy subjects ($P = 0.182$). As a result, we estimated mean DTI parameters (and 95% confidence intervals from the slice-wise standard deviation) across slices for the contralateral nerves in each TPNI subject/timepoint, which served as an internal control for comparison to the damaged/repared ipsilateral nerve in the same TPNI subject and timepoint. In other words, this allowed us to account for demographic factors (e.g., age, sex, body mass index) that may affect results across subjects as well as experimental factors that may affect measurements across time.

Cross-sectional analysis across cohorts with different injury severities

Figure 2 qualitatively summarizes the full range of values for each cohort, including the postsurgical data across all timepoints in the CTS and TPNI cohorts. Note the overall trend in the range of FA values, which were reduced in the CTS subjects relative to controls, and further reduced in the more severe TPNI subjects relative to the CTS subjects. For the quantitative statistical analysis, control data as well as TPNI/CTS data from each subject's first timepoint were compared via pair-wise Wilcoxon Rank-Sum tests. TPNI data showed no significant differences when compared with CTS for FA ($P = 0.156$), MD ($P = 0.461$), AD ($P = 0.683$), and RD values ($P = 0.461$). However, when compared with controls, TPNI showed significantly decreased FA ($P = 0.008$) and elevated MD

Table 2. Results of DTI metrics for each subject at each timepoint for each nerve. N/A refers to no surgery. CTS 5 had a diagnosis of carpal tunnel syndrome but had not yet received treatment at first scan. The units of mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) are $\mu\text{m}^2/\text{ms}$. Time listed in months since surgery

Subject	Time Postsurgery	State (Nerve)	FA $\pm$ SD	MD $\pm$ SD	AD $\pm$ SD	RD $\pm$ SD
TPNI 1 ²	1 month	Injured (Ulnar)	0.45 $\pm$ 0.11	1.08 $\pm$ 0.23	1.72 $\pm$ 0.31	0.77 $\pm$ 0.22
	N/A	Contralateral	0.57 $\pm$ 0.15	0.92 $\pm$ 0.18	1.64 $\pm$ 0.26	0.56 $\pm$ 0.19
TPNI 2	1 month	Injured (Median)	0.29 $\pm$ 0.05	1.39 $\pm$ 0.19	1.86 $\pm$ 0.24	1.16 $\pm$ 0.17
	N/A	Contralateral	0.55 $\pm$ 0.13	1.10 $\pm$ 0.25	1.90 $\pm$ 0.40	0.70 $\pm$ 0.20
	4 months	Injured (Median)	0.33 $\pm$ 0.07	1.45 $\pm$ 0.22	1.99 $\pm$ 0.30	1.18 $\pm$ 0.20
	N/A	Contralateral	0.53 $\pm$ 0.17	1.18 $\pm$ 0.34	2.00 $\pm$ 0.50	0.77 $\pm$ 0.31
	1 month	Injured (Ulnar)	0.33 $\pm$ 0.07	1.45 $\pm$ 0.17	1.99 $\pm$ 0.18	1.18 $\pm$ 0.19
	N/A	Contralateral	0.51 $\pm$ 0.17	1.12 $\pm$ 0.34	1.83 $\pm$ 0.49	0.76 $\pm$ 0.30
	4 months	Injured (Ulnar)	0.37 $\pm$ 0.10	1.36 $\pm$ 0.28	1.93 $\pm$ 0.34	1.07 $\pm$ 0.27
	N/A	Contralateral	0.60 $\pm$ 0.18	1.04 $\pm$ 0.29	1.93 $\pm$ 0.48	0.59 $\pm$ 0.23
TPNI 3	3 months	Injured (Ulnar)	0.31 $\pm$ 0.07	1.46 $\pm$ 0.22	1.97 $\pm$ 0.26	1.21 $\pm$ 0.22
	N/A	Contralateral	0.41 $\pm$ 0.13	1.15 $\pm$ 0.30	1.74 $\pm$ 0.46	0.85 $\pm$ 0.25
	6 months	Injured (Ulnar)	0.17 $\pm$ 0.06	1.81 $\pm$ 0.27	2.13 $\pm$ 0.32	1.65 $\pm$ 0.26
	N/A	Contralateral	0.32 $\pm$ 0.11	1.28 $\pm$ 0.36	1.78 $\pm$ 0.47	1.03 $\pm$ 0.32
	9 months ¹	Injured (Ulnar)	0.29 $\pm$ 0.12	1.29 $\pm$ 0.24	1.68 $\pm$ 0.27	1.09 $\pm$ 0.26
	N/A	Contralateral	0.36 $\pm$ 0.08	1.22 $\pm$ 0.21	1.75 $\pm$ 0.27	0.96 $\pm$ 0.20
Group:		Injured	0.32 $\pm$ 0.06	1.41 $\pm$ 0.15	1.91 $\pm$ 0.19	1.16 $\pm$ 0.15
		Contralateral	0.48 $\pm$ 0.14	1.13 $\pm$ 0.28	1.82 $\pm$ 0.42	0.78 $\pm$ 0.25
CTS 1	5 months	Compressed (Median)	0.45 $\pm$ 0.04	1.24 $\pm$ 0.08	1.88 $\pm$ 0.09	0.92 $\pm$ 0.09
	16 months	Compressed (Median)	0.46 $\pm$ 0.03	1.20 $\pm$ 0.05	1.87 $\pm$ 0.05	0.87 $\pm$ 0.06
	20 months	Compressed (Median)	0.41 $\pm$ 0.03	1.10 $\pm$ 0.07	1.59 $\pm$ 0.07	0.85 $\pm$ 0.08
	24 months	Compressed (Median)	0.46 $\pm$ 0.03	1.20 $\pm$ 0.05	1.87 $\pm$ 0.05	0.87 $\pm$ 0.06
CTS 2	4.5 months	Compressed (Median)	0.43 $\pm$ 0.03	1.37 $\pm$ 0.05	2.04 $\pm$ 0.06	1.03 $\pm$ 0.06
	13.5 months	Compressed (Median)	0.35 $\pm$ 0.02	1.27 $\pm$ 0.04	1.77 $\pm$ 0.04	1.02 $\pm$ 0.05
	16.5 months	Compressed (Median)	0.43 $\pm$ 0.02	1.15 $\pm$ 0.04	1.74 $\pm$ 0.05	0.85 $\pm$ 0.04
CTS 3	1 month	Compressed (Median)	0.41 $\pm$ 0.02	1.23 $\pm$ 0.04	1.84 $\pm$ 0.04	0.93 $\pm$ 0.05
	12 months	Compressed (Median)	0.43 $\pm$ 0.02	1.33 $\pm$ 0.04	2.01 $\pm$ 0.06	0.99 $\pm$ 0.05
	16 months	Compressed (Median)	0.41 $\pm$ 0.02	1.20 $\pm$ 0.19	1.80 $\pm$ 0.28	0.90 $\pm$ 0.17
CTS 4	4 months	Compressed (Median)	0.46 $\pm$ 0.02	1.23 $\pm$ 0.05	1.90 $\pm$ 0.07	0.89 $\pm$ 0.05
	14 months	Compressed (Median)	0.42 $\pm$ 0.04	1.32 $\pm$ 0.07	1.95 $\pm$ 0.07	1.00 $\pm$ 0.08
CTS 5	<1 month	Compressed (Median)	0.51 $\pm$ 0.04	1.24 $\pm$ 0.08	1.97 $\pm$ 0.08	0.88 $\pm$ 0.09
	3.5 months	Compressed (Median)	0.43 $\pm$ 0.04	1.31 $\pm$ 0.06	1.94 $\pm$ 0.05	0.99 $\pm$ 0.07
CTS 6	1 month	Compressed (Median)	0.44 $\pm$ 0.01	1.36 $\pm$ 0.02	2.09 $\pm$ 0.04	0.99 $\pm$ 0.03
	14 months	Compressed (Median)	0.45 $\pm$ 0.02	1.28 $\pm$ 0.04	1.98 $\pm$ 0.06	0.93 $\pm$ 0.04
CTS 7	6 months	Compressed (Median)	0.30 $\pm$ 0.04	1.47 $\pm$ 0.08	1.87 $\pm$ 0.10	1.28 $\pm$ 0.09
CTS 8	2 months	Compressed (Median)	0.46 $\pm$ 0.03	1.23 $\pm$ 0.05	1.91 $\pm$ 0.04	0.89 $\pm$ 0.06
Group:			0.43 $\pm$ 0.03	1.27 $\pm$ 0.06	1.90 $\pm$ 0.06	1.01 $\pm$ 0.06
Control 1	N/A	Healthy (Median)	0.54 $\pm$ 0.03	1.13 $\pm$ 0.04	1.91 $\pm$ 0.04	0.74 $\pm$ 0.05
Control 2	N/A	Healthy (Median)	0.39 $\pm$ 0.03	1.25 $\pm$ 0.05	1.82 $\pm$ 0.05	0.96 $\pm$ 0.06
Control 3	N/A	Healthy (Median)	0.54 $\pm$ 0.02	1.07 $\pm$ 0.06	1.79 $\pm$ 0.05	0.71 $\pm$ 0.06
Control 4	N/A	Healthy (Median)	0.61 $\pm$ 0.03	1.04 $\pm$ 0.05	1.86 $\pm$ 0.05	0.62 $\pm$ 0.05
Control 5	N/A	Healthy (Median)	0.64 $\pm$ 0.03	1.09 $\pm$ 0.08	1.99 $\pm$ 0.09	0.64 $\pm$ 0.08
Control 6	N/A	Healthy (Median)	0.70 $\pm$ 0.02	0.95 $\pm$ 0.03	1.89 $\pm$ 0.04	0.49 $\pm$ 0.04
Control 7	N/A	Healthy (Median)	0.64 $\pm$ 0.04	1.07 $\pm$ 0.04	2.00 $\pm$ 0.03	0.60 $\pm$ 0.06
Control 8	N/A	Healthy (Median)	0.66 $\pm$ 0.03	1.11 $\pm$ 0.03	2.10 $\pm$ 0.03	0.61 $\pm$ 0.04
Group:			0.59 $\pm$ 0.03	1.09 $\pm$ 0.05	1.92 $\pm$ 0.05	0.67 $\pm$ 0.05

¹Indicates measures acquired after second surgery in TPNI 3.

²Indicates knee coil was used during MRI acquisition for TPNI 1.

($P = 0.048$) and RD values ($P = 0.008$). Additionally, comparing CTS to controls also showed decreased FA ($P = 0.007$) and elevated MD ($P = 0.003$) and RD values ($P = 0.003$). There were no significant differences in AD

for any of these cohort comparisons ($P > 0.808$). It should be noted that while this analysis includes only the first timepoints for both TPNI and CTS groups, there were no significant changes over timepoints after surgery

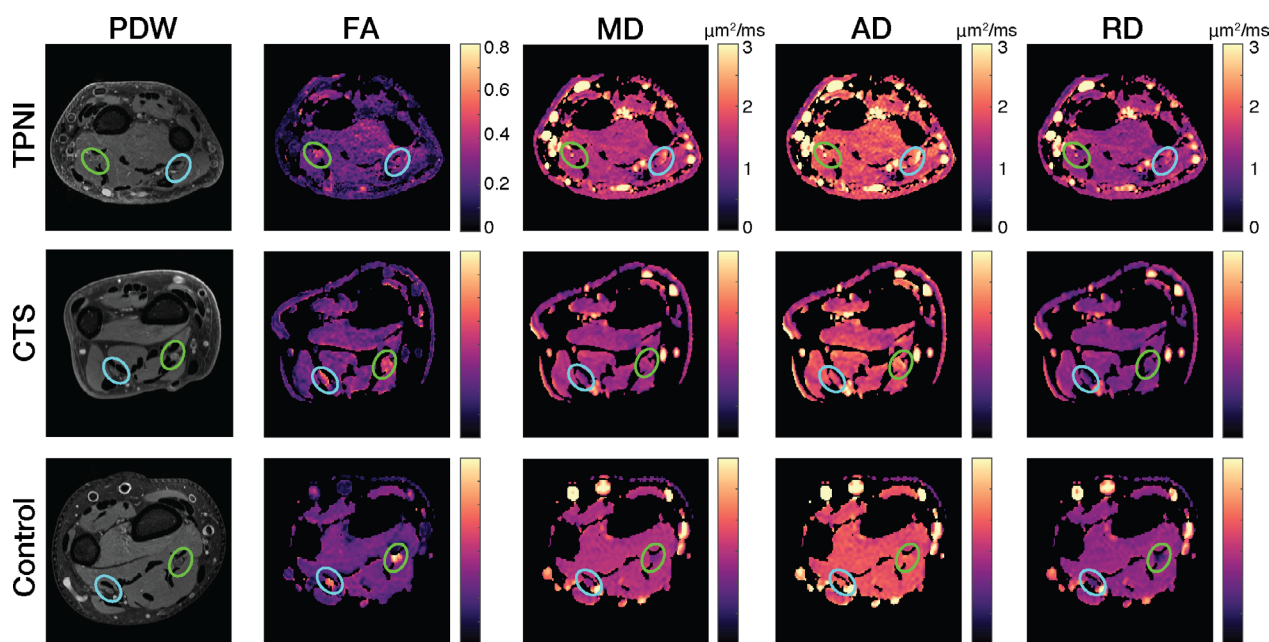


Figure 1. Representative Images: Proton density-weighted (PDW), fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps show for traumatic peripheral nerve injury cohort (TPNI), carpal tunnel syndrome cohort (CTS), and control cohort. Green circles indicate the median nerve. Blue circles represent the ulnar nerve.

for CTS subjects (Spearman rank correlation coefficient = 0.198, $P = 0.430$). Furthermore, it is worth noting that more variability was present for DTI parameters from TPNI than for the healthy controls, which is likely related to the variability of injuries in this cohort and the combined use of measurements in the proximal and distal slices.

Cross-sectional analysis of injured & contralateral nerve in TPNI

TPNI data at each timepoint and nerve injury were used in this analysis, resulting in eight paired data points from three subjects. At the group level, a Wilcoxon Signed-Rank analysis of DTI metrics showed injured nerves had significantly lower FA values than contralateral nerves ($P = 0.008$), significantly elevated MD ($P = 0.008$) and RD values ($P = 0.008$), and similar AD values ($P = 0.250$). These results are illustrated in Figure 3.

The difference in measures between the injured and contralateral nerve is further illustrated for a single subject in Figure 4, where lower FA values are present in the injured ulnar nerve, and higher FA values in the contralateral ulnar nerve in both proximal and distal images. Note that while Figure 4 shows images of TPNI 3, this phenomenon was observed in the other two TPNI subjects as well.

Subject TPNI 1

In the first subject, TPNI 1, a comparison of FA between the right arm with the repaired median nerve to the left arm with the uninjured median nerve was shown to be significantly different ($P = 0.021$). Figure 5 shows the slice-wise measurements for FA in the injured and uninjured median nerves of Subject 1, with more proximal slices (slice 1-3) of the injured nerve within the boundary of the measures observed in the healthy nerve. As the images move more distal towards the injury site in slices (4-7), the FA values then fall outside the confidence intervals, with the lowest measure being at slice 6 and continuing upwards with more distal slices. Figure 6 visualizes these data through diffusion fibertracking of the injured median nerve in the right arm, showing the site of injury and how FA values drop distally from the injury toward the hand.

Subject TPNI 2

In the second subject, TPNI 2, a comparison of the FA of the i) right arm, injured median and ulnar nerves, to the ii) left arm, uninjured median and ulnar nerves, showed significant differences across both longitudinal timepoints ($P < 0.001$, in all cases), with FA being lower in the injured nerve. Figure 7 shows the slice-wise measurements

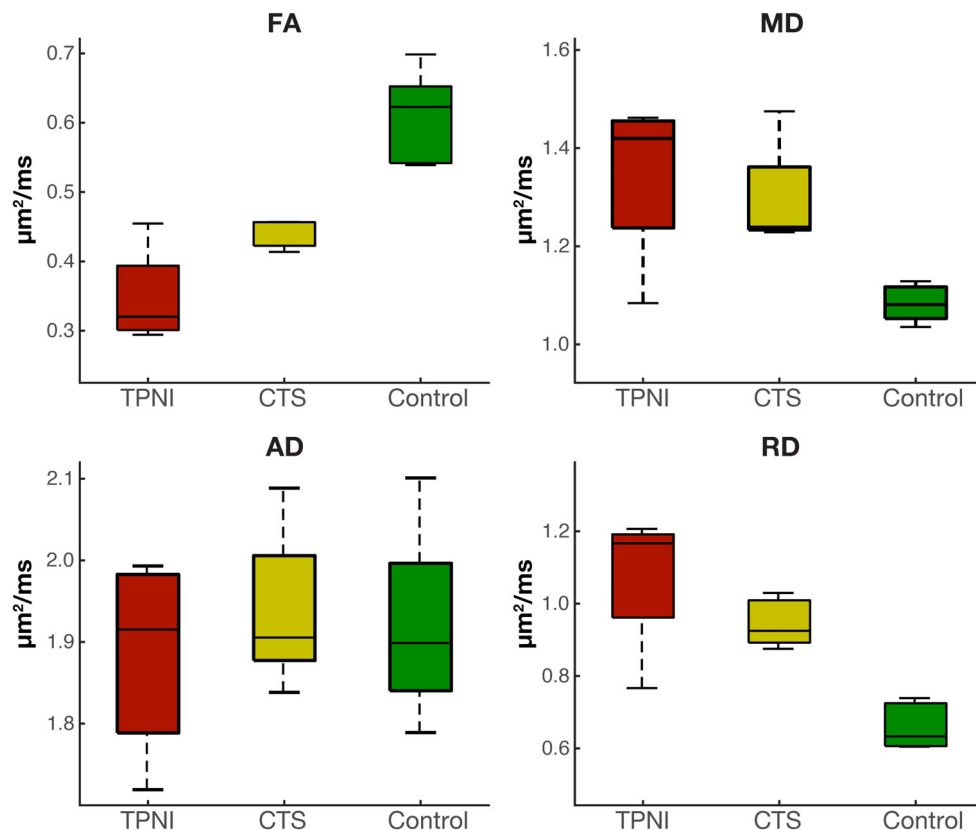


Figure 2. Group comparison of each DTI metric for the traumatic peripheral nerve injury (TPNI: red, $n = 8$), carpal tunnel syndrome (CTS: yellow, $n = 18$), and control cohorts (green, $n = 8$) across all subjects and timepoints. The black central mark represents the median, the edges of the box are the 25th and 75th percentile, and the whiskers show the interquartile range beyond these edges. This includes all longitudinal data for 3 TPNI and 8 CTS subjects.

for FA in the injured and uninjured median and ulnar nerves of TPNI 2. From 1-month postsurgery to 4 months postsurgery, the injured nerve data fall well below the range of healthy nerve in the contralateral arm; however, there were overall increases of FA in the injured nerve from scan 1 to scan 2, indicating a return toward healthy values over time. It should be noted that this recovery was aided by the PEG treatment. Furthermore, the observed increase in FA across time agreed with the clinical findings in Table 1, which indicated sensorimotor improvement over the injured arm over the same period.

Subject TPNI 3

Once again, FA was significantly lower in injured nerves compared to the contralateral, healthy nerves over all three timepoints in TPNI 3 ($P < 0.001/P < 0.001/P = 0.028$). Figure 8 shows slice-wise measures for FA in the injured and contralateral healthy ulnar nerve in TPNI 3. The reduction in FA values at 6 months related to

3 months were consistent with clinical findings reported in Table 1, which indicated an unsuccessful initial repair. Following a secondary repair procedure, FA values overlapped the 95% CI of the contralateral nerve by 9 months (relative to the first procedure), which was once again consistent with clinical findings and is indicative of a successful re-operation with a nerve graft. In summary, clinical measures showed a decline from the first timepoint to the second timepoint, which agreed with the DTI measures we acquired at these timepoints. In addition to this, clinical measures showed an improvement from the second timepoint to the third timepoint, which indicated the re-operation between the second and third timepoint was successful, which is reflected in our DTI results as an increased FA. Overall, lower FA were observed in this subject, which exemplifies why having data from the contralateral arm is important in these kinds of studies to correct for potentially confounding demographic factors such as age and body mass index (See: Table 1, TPNI 3).

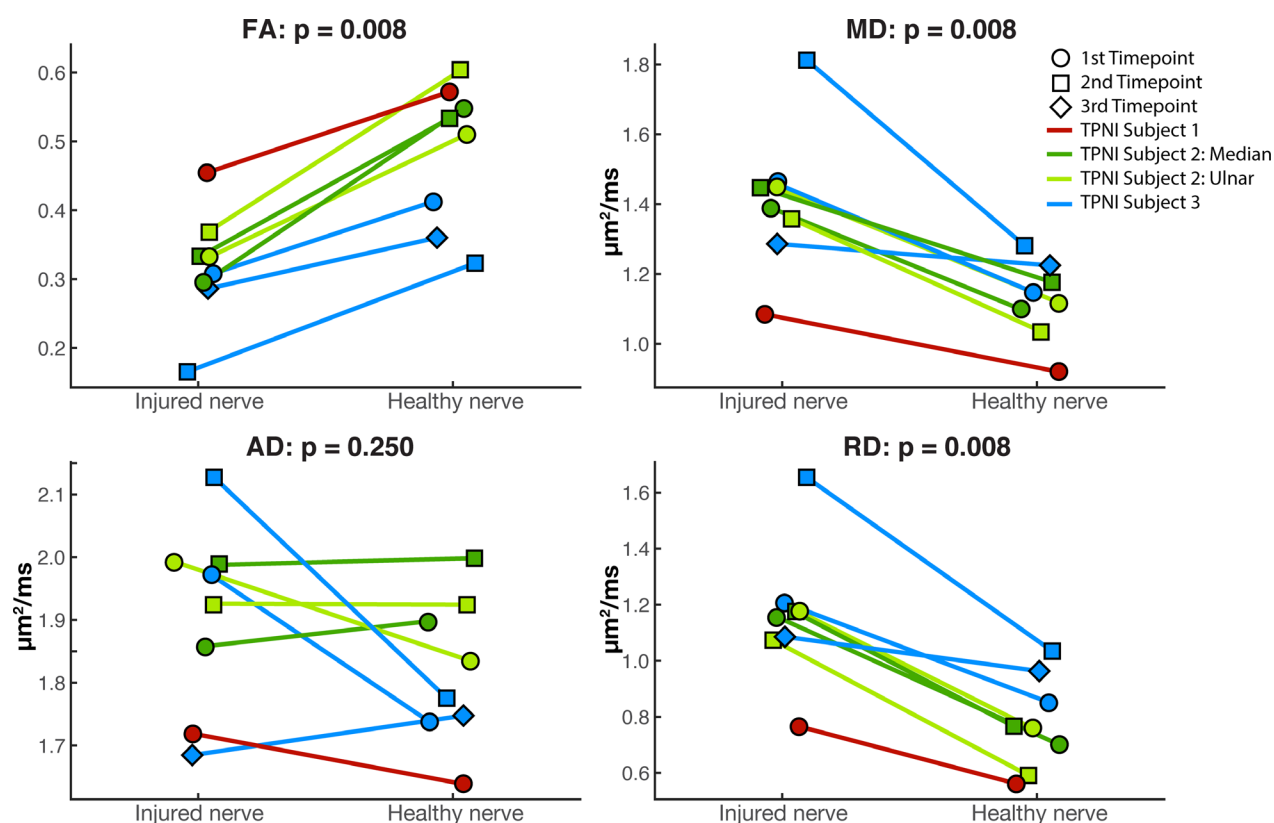


Figure 3. Results of all TPNI subjects ($n = 3$) and timepoints comparing injured nerve data to the contralateral healthy nerve data for all DTI metrics acquired: FA (top-left), MD (top-right), AD (bottom-left), and RD (bottom-right). Circles represent the first timepoint, squares represent the second timepoint (TPNI 2 & 3), and diamond represents the third timepoint (TPNI 3). Red lines represent TPNI 1, green lines represent TPNI 2 (dark/light = median/ulnar), and blue represents TPNI 3. P -values of Wilcoxon Signed-Rank test are shown above each boxplot.

Discussion

In this small case-control study, we demonstrated that noninvasive DTI measures are sensitive to traumatic nerve injury and nerve regeneration over time. While the clinical measures do reflect the degree of deficits following TPNI, they are not specific to the degree of injury, which may limit the ability of surgeons to determine when to re-operate. Across all subjects, i) FA values were significantly reduced in TPNI and CTS cohorts relative to healthy control nerves and ii) DTI findings were consistent with changes in clinical measures across the same periods, suggesting these measures may be indicative of nerve degeneration and regeneration. More specifically, we found that DTI measures improved after a re-operation procedure (due to a lack of sensorimotor improvement after the first procedure) in one subject (TPNI 3) and after repair-promoting PEG treatment in another patient (TPNI 2). Together, these findings demonstrate the feasibility of performing DTI in the nerves of the wrist after trauma and surgical repair. Furthermore, these

measures may provide a noninvasive and reliable assessment of regeneration, which may improve clinical decision-making and possibly alter outcomes after surgical interventions.

TPNI 1 suffered a right arm median nerve injury and showed large differences for FA between the injured median nerve compared to the uninjured median nerve of the right arm. These results indicate that DTI can differentiate between injured and uninjured nerves. TPNI 2 suffered a median and ulnar nerve injury at the forearm of the right arm. At the first timepoint, TPNI 2 showed significant differences between both the injured median and ulnar nerves of the right arm when compared with the contralateral uninjured median and ulnar nerves of the left arm. This trend continued three months later at the second timepoint, where significant differences between nerves and arms were observed. For the median nerve, this is conceivably due to the PEG gel that this subject received. For the ulnar nerve, it was expected that the recovery would be more complete, as this nerve experienced a partial transection (the median nerve was fully

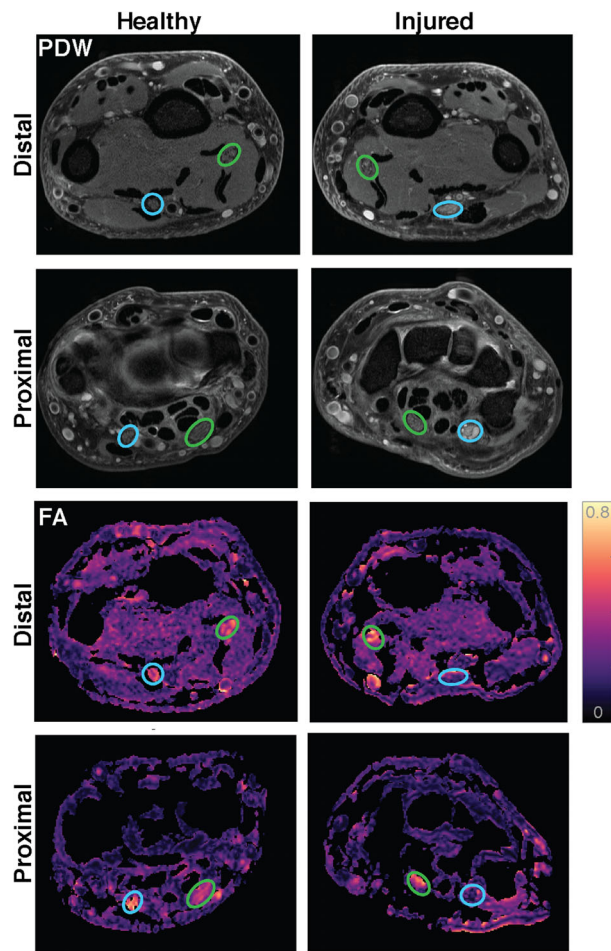


Figure 4. Proton density-weighted (PDW) and fractional anisotropy (FA) maps for injured and healthy nerves in TPNI 3. Green circles indicate the median nerve. Blue circles represent the ulnar nerve. Worth nothing are the differences in intensity between the injured ulnar nerve and healthy ulnar nerve.

transected). As these results indicate, DTI can also show improvement over time. TPNI 3 suffered a left arm, ulnar nerve injury that was captured with longitudinal MRI. At the first timepoint, there was a clear delineation between healthy and injured ulnar measures for FA, similar to what was observed in the previous two subjects. At the following timepoint, however, mean FA measures decreased. This agreed with the clinical assessment, which indicated that the initial repair had failed. This subject was brought back to the operating room and a revision was performed. Three months later, FA values increased above what was observed at the first timepoint, indicating that the re-repair was successful. Therefore, the MRI findings showed that before revision this subject was not healing. The failure rate on nerve repairs is approximately 40%, and this case is an example of how monitoring

recovery from nerve trauma can inform the decision to surgically re-intervene. The ability to identify these failures early, re-operate, and evaluate over time if the second intervention is working is the ideal application of DTI in monitoring recover after nerve trauma.

These longitudinal findings also indicate that recovery is not homogeneous between subjects. Considering that these traumatic injuries occur in a variety of ways, the rate of recovery can be dramatically different between subjects due to a multitude of factors. Factors such as nutrition, smoking, comorbidities, or, in some cases after injury, overexertion during recovery can slow the healing process. Given this heterogeneity, the objective measures provided by DTI may improve clinical decision-making by identifying cases that require re-operation earlier than existing methods, although additional data in larger cohorts are required to evaluate this claim.

Observed FA changes are driven by RD rather than AD

In these results, it is apparent that RD is the biggest factor affecting the apparent changes in FA in the TPNI subjects. Studies have shown that RD is reflective of myelin integrity and axon density as it measures diffusion perpendicular to the axon, whereas AD is reflective of acute axonal degeneration as it measures diffusion along the axon.^{37,38} As TPNI subjects experience remyelination and axonal sprouting over time, we might anticipate that differences in RD between injured and healthy nerve would arise, as we observed when comparing TPNI with controls ($P = 0.008$), due to the reduced myelination and/or axonal density in recovering nerves. In TPNI, axonal sprouting occurs primarily before secondary remyelination to establish a reconnection from the axon to its target muscle or sensor after damaged tissue has been removed via the process of Wallerian degeneration.³⁹ However, it is difficult to separate axonal regeneration from remyelination using measures of RD alone, and a more myelin-specific measure, like magnetization transfer MRI,⁴⁰ could further improve specificity. For AD, previous animal studies have shown that AD is sensitive to axonal regeneration in the first two weeks after injury.⁴¹ However, we acquired MRI data later within our cohort (i.e., after Wallerian degeneration), potentially explaining why we did not observe any significant differences for AD between injured and contralateral, healthy nerves ($P = 0.250$).

Another important factor in evaluating the relationship between DTI metrics and underlying tissue microstructure is the influence of inflammation and edema.⁴² In these kinds of injuries, it would be expected to see edema and/or inflammation occur around the injury site, and these

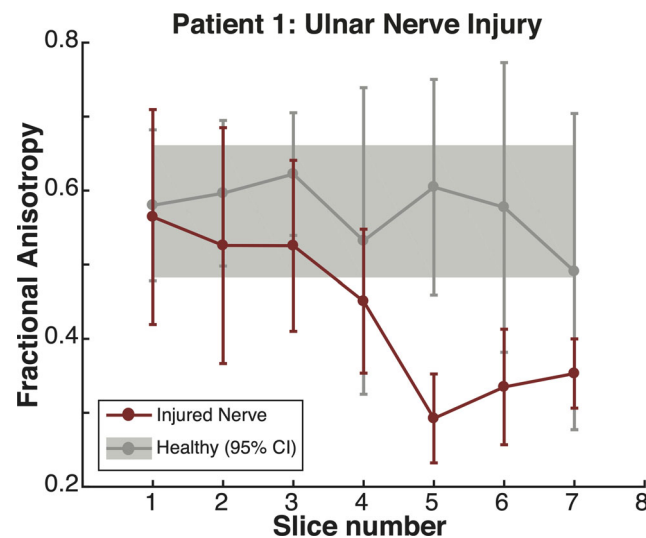


Figure 5. Results for TPNI 1: Fractional anisotropy plotted by slice. Red errorbars reflect individual data points of the injured ulnar nerve, with bars showing slice-wise standard deviations. Gray errorbars reflect analogous information in contralateral healthy ulnar nerve, with the shaded gray area representing the 95% confidence interval (CI) for averaged healthy ulnar nerve across all slices.

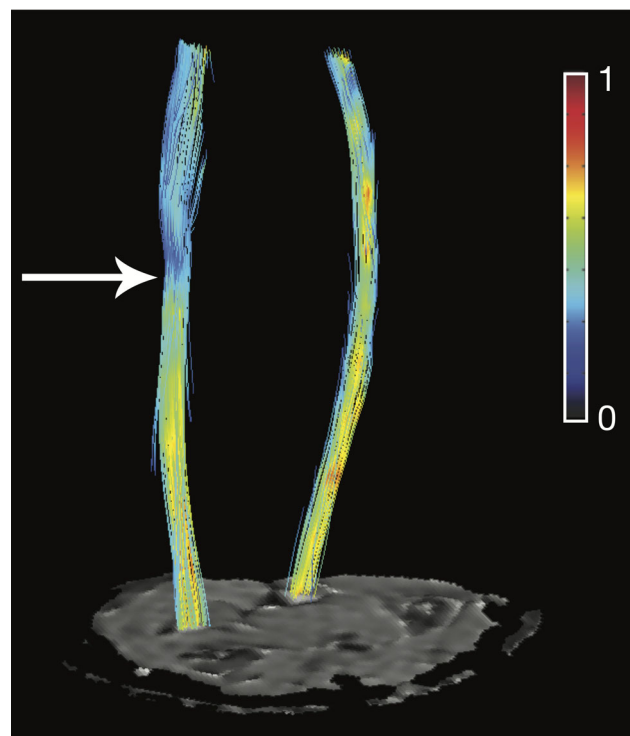


Figure 6. Fibertracking results of TPNI 1 with a right arm median nerve injury. A proximal slice is shown at the bottom of the image with ascending fiber tracking of the injured ulnar nerve (left) and healthy median nerve (right). The white arrow indicates the area of injury. The nerve is color-coded for fractional anisotropy (FA).

effects would diminish over time during the healing process. For these reasons, we would also expect MD to increase with the presence of edema.⁴³ The lingering

effects of edema over healing may reflect the observed differences we see for MD in comparing TPNI injured and contralateral nerves ($P = 0.008$). In addition, these

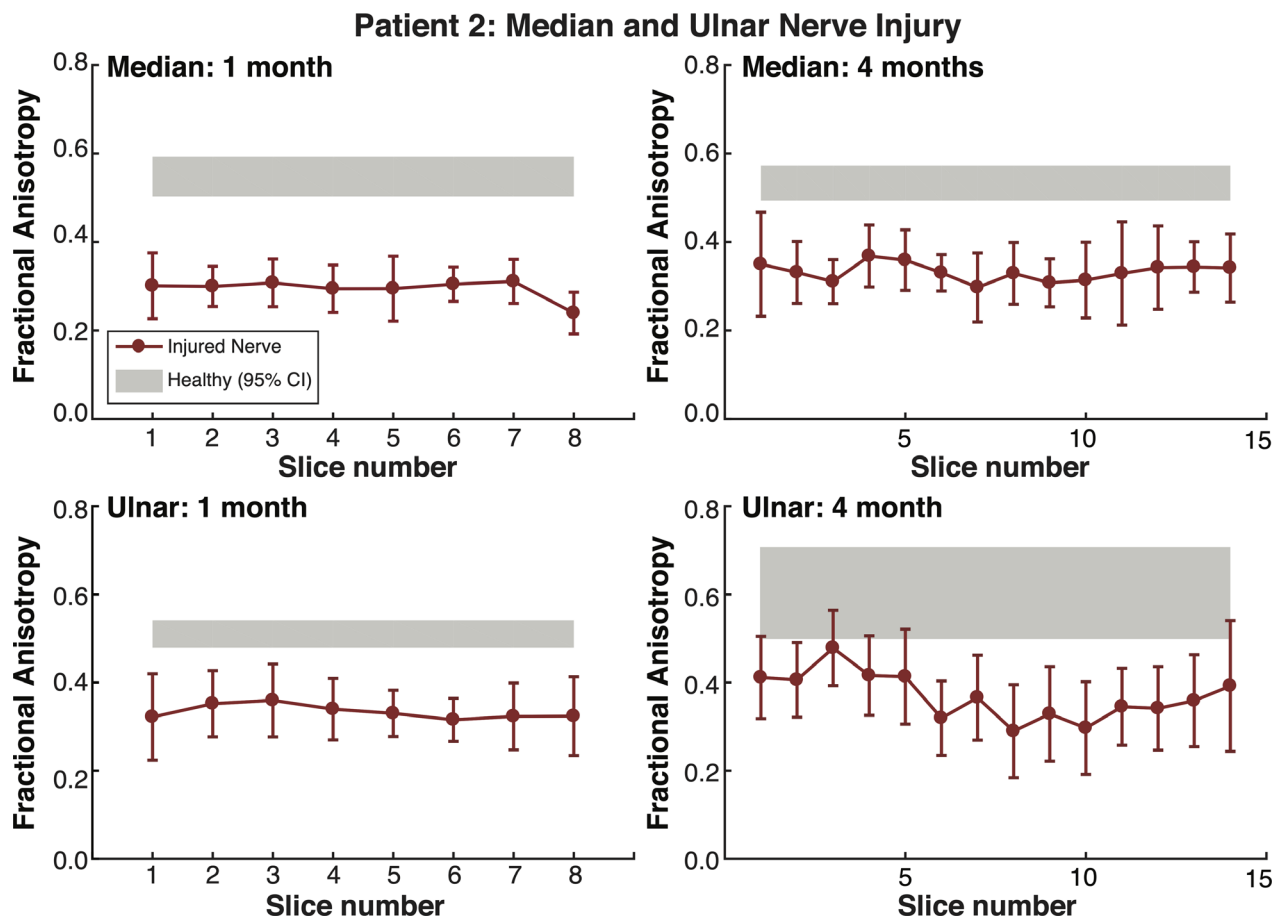


Figure 7. Results for TPNI 2: FA plotted by slice. Red errorbars reflect individual data points of the injured nerves, with bars showing slice-wise standard deviations. The gray shaded gray area represents the 95% confidence interval (CI) for averaged healthy nerve data in the contralateral arm. The top charts show measures for median nerve across two timepoints. Likewise, the bottom charts show measures for the ulnar nerve across the same timepoints. The ulnar nerve was only partially transected and showed superior recovery, whereas the median nerve was fully transected and showed less recovery by the second timepoint.

processes likely also impact FA measurements, as edema is expected to increase diffusion isotropy.

Comparison to milder injury and healthy controls

The comparison of TPNI, CTS, and healthy subjects showed that DTI was sensitive to the degree of injury across all cohorts. Interestingly, this comparison used the data points acquired from all timepoints, which could also be considered as the range of observed values over the time course for healing. In the TPNI cohort, we saw two of the three subjects improve over time, indicating that the nerve repair was successful to allow for regeneration. However, in the CTS cohort, we did not observe a significant change in FA over time after CTS release surgery. This may be due to the fact the CTS release surgery is not as invasive because the surgery decompresses rather

than reconnects detached nerves. In TPNI surgeries, proximal and distal portions of the injured nerve ends are reconnected, with all the damaged tissue removed to allow proper healing. However, in CTS release surgery, only the carpal tunnel ligament is cut, which frees up room around the nerve and relieves compression. Additionally, the timepoints we observed CTS subjects may have, in some cases, been after a full recovery, as the time course of full recovery for CTS release surgery is typically 4–6 months.⁴⁴ Finally, in the control group, higher values for FA than in CTS, which is consistent with recent literature.^{45–48}

Limitations

It is worth mentioning the limitations of this study for accurate interpretations of the findings. We obtained a small cohort, and as such, chose to analyze the data

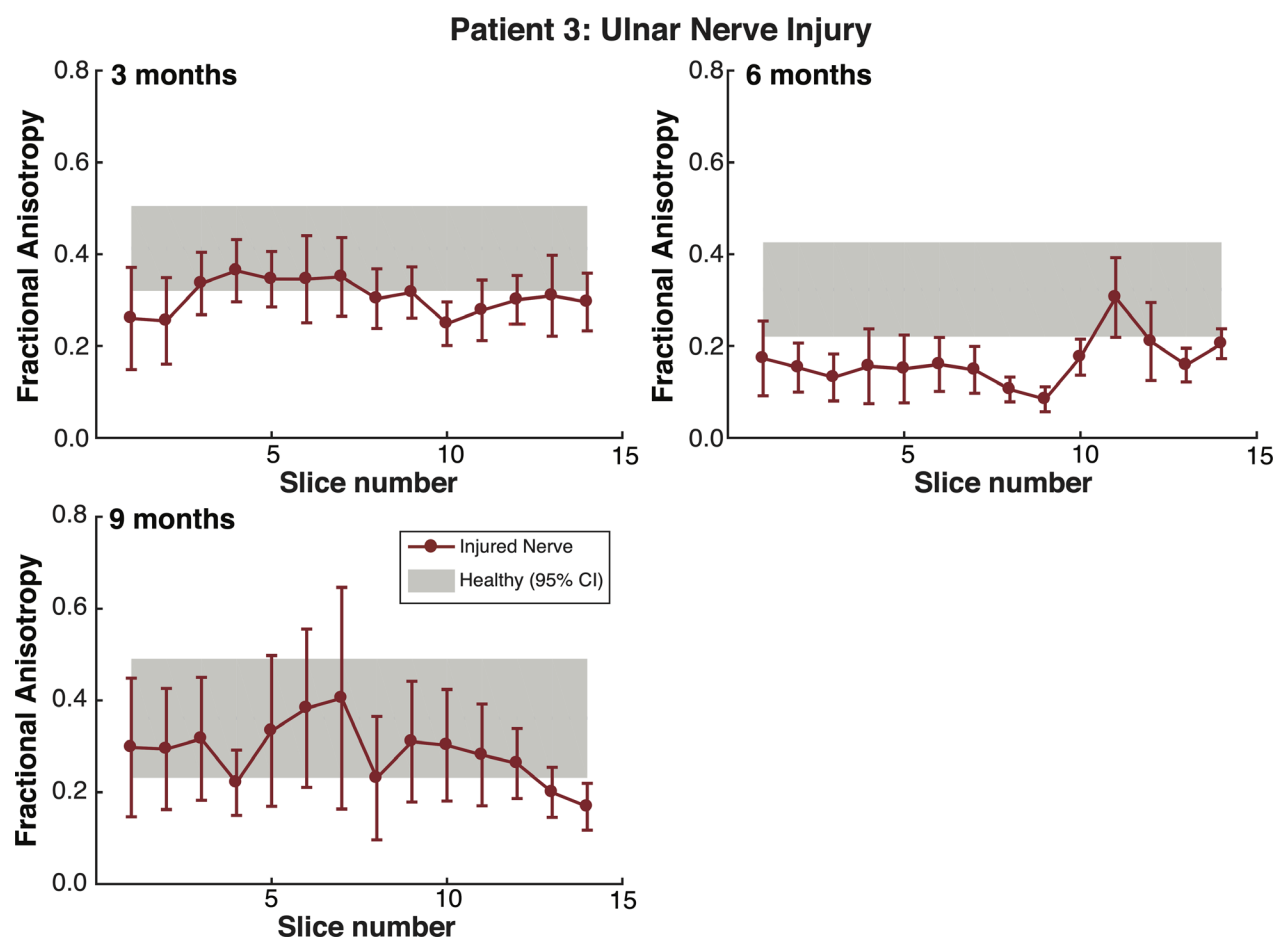


Figure 8. Results for TPNI 3: Fractional Anisotropy plotted by slice. The red errorbars reflect individual data points of the injured ulnar nerve, with bars showing slice-wise standard deviations. The gray shaded gray area represents the 95% confidence interval (CI) for averaged healthy ulnar nerve data in the contralateral arm. The top charts show measures across two timepoints, 3 and 6 months, whereas the bottom chart shows measures acquired at the third timepoint at 9 months postsurgery. At 6 months, FA decreased which mirrored clinical data showing failure. This led to re-repair at 9 months and FA recovered.

mostly on an individual subject basis. In addition, the affected nerve (median and/or ulnar), injury severity (full/partial transection), and treatment (PEG, reoperation) varied across TPNI subjects, all of which have a significant impact on outcomes. For these reasons, we expect differences in recovery rate. To overcome these limitations, we analyzed each TPNI subject individually, comparing results in the injured nerve(s) to the contralateral arm on a per subject basis for only the TPNI cohort. In addition, TPNI subjects showed greater variability in contralateral nerve DTI measures, which may be explained by subject comfort diminishing over time and related motion artifacts. This effect was also present in the subset of CTS subjects that received bilateral scans (data not shown). Given these findings, future studies will focus on methods that maximize subject comfort and minimize scan time. For example, we will investigate

methods that allow for supine scanning that overcome issues with distortion that are increased in this position. Finally, the CTS and control groups were not age- and sex-matched, which if done correctly would lead to greater generalizable findings and eliminate the possibility of confounding variables. Future work will focus on developing study designs that account for these limitations. Also broadening the timepoints of the study may provide additional information on recovery as traumatic injury and carpal tunnel syndrome recover at different rates. A larger pool of TPNI subjects will also allow us to group by types of injury (e.g., full vs. partial laceration) and by which nerve(s) was damaged (median, ulnar, both). Additionally, clinical measures and presurgical data should be utilized in future studies to evaluate the prognostic value of DTI indices and to better characterize heterogeneity with each cohort (TPNI and/or CTS).

Acknowledgments

The authors thank Marcia Spear, Ellie Dahms, and Alesia Pruitt for outreach, subject recruitment, and study coordination. The authors also thank the MRI technologists at VUIIS for their ongoing support and expertise. This research was supported by grants from the National Institute of Health/National Institute of Neurological Disease and Stroke R01 NS097821 (RDD) and Department of Defense W81XWH-15-JPC-8/CRM RP-NMSIRA, MR150075 (WPT).

Authors' Contributions

M.D.P.: drafting/revising the manuscript, study concept and design, analysis and interpretation of data, acquisition of data, and statistical analysis; G.E.G.: drafting/revising the manuscript, acquisition of data; A. C. P.: drafting/revising the manuscript, acquisition of data, interpretation of data. I.V.M.E.: interpretation of data and data analysis. G.P.: drafting/revising the manuscript. B. C. D.: drafting/revising the manuscript, study concept and design. D.R.W.: drafting/revising the manuscript, study design and concept. M.D.D.: drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, and study supervision; W.P.T.: drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, and study supervision; R.D.D.: drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, funding support, and study supervision.

Conflict of Interest

No authors in this manuscript have any conflict of interest to disclose.

REFERENCES

- Campbell WW. Evaluation and management of peripheral nerve injury. *Clin Neurophysiol* 1978; <https://doi.org/10.1016/j.clinph.2008.03.018>.
- Noble J, Munro CA, Prasad VSSV, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma - Inj Infect Crit Care* 1998;45:116–122.
- Chhabra A, Ahlawat S, Belzberg A, Andreseik G. Peripheral nerve injury grading simplified on MR neurography: As referenced to Seddon and Sunderland classifications. *Indian J Radiol Imaging* 2014;24:217.
- Cross JD, Ficke JR, Hsu JR, et al. Battlefield orthopaedic injuries cause the majority of long-term disabilities. *J Am Acad Orthop Surg* 2011;19:1–7.
- Stansbury LG, Branstetter JG, Lalliss SJ. Amputation in military trauma surgery. *J Trauma - Inj Infect Crit Care* 2007;63:940–944.
- Ciaramitaro P, Mondelli M, Logullo F, et al. Traumatic peripheral nerve injuries: Epidemiological findings, neuropathic pain and quality of life in 158 patients. *J Peripher Nerv Syst* 2010;15:120–127.
- Kouyoumdjian JA. Peripheral nerve injuries: a retrospective survey of 456 cases. *Muscle Nerve* 2006;34:785–788.
- Brown JM, Tung THH, Mackinnon SE. Median to radial nerve transfer to restore wrist and finger extension. *Oper Neurosurg* 2010;66:ons75–ons83.
- Mafi P, Hindocha S, Dhital M, Saleh M. Advances of peripheral nerve repair techniques to improve hand function: a systematic review of literature. *Open Orthop J* 2012;6:60–68.
- Ruijs ACJ, Jaquet JB, Kalmijn S, et al. Median and ulnar nerve injuries: A meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair. *Plast Reconstr Surg* 2005;116:484–494.
- Lee DH, Claussen GC, Oh S. Clinical nerve conduction and needle electromyography studies. *J Am Acad Orthop Surg* 2004;12:276–287.
- Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int* 2014;2014.
- Chaudhry V, Cornblath DR. Wallerian degeneration in human nerves: Serial electrophysiological studies. *Muscle Nerve* 1992;15:687–693.
- Oh SJ. Electromyographic studies in peripheral nerve injuries. *South Med J* 1976;69:177–182.
- Robinson LR. Traumatic injury to peripheral nerves. *Muscle Nerve* 2000;23:863–873.
- Cartwright MS, Chloros GD, Walker FO, et al. Diagnostic ultrasound for nerve transection. *Muscle Nerve* 2007;35:796–799.
- Suk JI, Walker FO, Cartwright MS. Ultrasound of peripheral nerves. *Curr Neurol Neurosci Rep* 2013;13:328.
- Filler AG, Kliot M, Howe FA, et al. Application of magnetic resonance neurography in the evaluation of patients with peripheral nerve pathology. *J Neurosurg* 1996;85:299–309.
- Behr B, Schnabel R, Mirastschijski U, et al. Magnetic resonance imaging monitoring of peripheral nerve regeneration following neurotmesis at 4.7 tesla. *Plast Reconstr Surg* 2009;123:1778–1788.
- Agosta F, et al. In vivo assessment of cervical cord damage in MS patients: A longitudinal diffusion tensor MRI study. *Brain* 2007;130:2211–2219.
- Bennett RE, Mac Donald CL, Brody DL. Diffusion tensor imaging detects axonal injury in a mouse model of repetitive closed-skull traumatic brain injury. *Neurosci Lett* 2012;513:160–165.

22. Filippi M, Cercignani M, Inglese M, Al E. Diffusion tensor magnetic resonance imaging. *Neurology* 2001;56:304–311.
23. Heckel A, et al. Peripheral nerve diffusion tensor imaging: assessment of axon and myelin sheath integrity. *PLoS One* 2015;10:e0130833.
24. Jeon T, Fung MM, Koch KM, et al. Peripheral nerve diffusion tensor imaging: Overview, pitfalls, and future directions. *J Magn Reson Imaging* 2018;47:1171–1189.
25. Meek MF, Stenekes MW, Hoogduin HM, Nicolai JA. In vivo three-dimensional reconstruction of human median nerves by diffusion tensor imaging. *Exp Neurol* 2006;198:479–482.
26. Kabakci NT, Kovanlikaya A, Kovanlikaya I. Tractography of the Median Nerve. *Semin Musculoskelet Radiol* 2009;198:479–482.
27. Zhou Y, Narayana PA, Kumaravel M, et al. High resolution diffusion tensor imaging of human nerves in forearm. *J Magn Reson Imaging* 2014;39:1374–1383.
28. Awdeh H, Wadhwa V, Andreisek G, et al. Diffusion Tensor Imaging of Peripheral Nerves. *Semin Musculoskelet Radiol* 2015;19:191–200.
29. Mori S, Van Zijl PCM. Fiber tracking: principles and strategies - a technical review. *NMR Biomed* 2002;15:468–480.
30. Boyer RB, et al. 4.7-T diffusion tensor imaging of acute traumatic peripheral nerve injury. *Neurosurg Focus* 2015;39:E9.
31. Farinas AF, Narayana PA, Kumaravel M, et al. Diffusion tensor tractography to visualize axonal outgrowth and regeneration in a 4-cm reverse autograft sciatic nerve rabbit injury model. *Neurol Res* 2019;41:257–264.
32. Ghergherehchi CL, Mikes M, Sengelaub DR, et al. Polyethylene glycol (PEG) and other bioactive solutions with neurorrhaphy for rapid and dramatic repair of peripheral nerve lesions by PEG-fusion. *J Neurosci Methods* 2019;314:1–12.
33. Bamba R, Waitayawinyu T, Nookala R, et al. A novel therapy to promote axonal fusion in human digital nerves. *J Trauma Acute Care Surg* 2016;81:S177–S183.
34. Paskal AM, Paskal W, Pietruski P, Wlodarski PK. Polyethylene glycol: The future of posttraumatic nerve repair? systemic review. *Int J Mol Sci* 2019;20:1–10.
35. Zhou Y, Kumaravel M, Patel VS, et al. Diffusion tensor imaging of forearm nerves in humans. *J Magn Reson Imaging* 2012;36:920–927.
36. Bazina P-L, Cuzzocreo JL, Yassa MA, et al. Volumetric neuroimage analysis extensions for the MIPAV software package. *J Neurosci Methods* 2007;165:111–121.
37. Song SK, Sun SW, Ju WK, et al. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *NeuroImage* 2003;20:1714–1722.
38. Morisaki S, Kawai Y, Umeda M, et al. In vivo assessment of peripheral nerve regeneration by diffusion tensor imaging. *J Magn Reson Imaging* 2011;33:535–542.
39. Svenngisen ÅF, Dahlin LB. Repair of the peripheral nerve-remyelination that works. *Brain Sci* 2013;3:1182–1197.
40. Chen Y, Haacke EM, Li J. Peripheral nerve magnetic resonance imaging. *F1000Research* 2019;8:1803.
41. Manzanera Esteve IV, Farinas AF, Pollins AC, et al. Probabilistic assessment of nerve regeneration with diffusion MRI in rat models of peripheral nerve trauma. *Sci Rep* 2019;9:1–11.
42. Winkowski PJ, Sabisz A, Naumczyk P, et al. Understanding the physiopathology behind axial and radial diffusivity changes-what do we know? *Front Neurol* 2018;9.
43. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion Tensor Imaging of the Brain Andrew. *Neurotherapeutics* 2007;4:316–329.
44. Rotman MB, Enkvetchakul BV, Megerian JT, Gozani SN. Time course and predictors of median nerve conduction after carpal tunnel release. *J Hand Surg Am* 2004;29:367–372.
45. Wang H, Ma J, Zhao L, et al. Utility of MRI diffusion tensor imaging in carpal tunnel syndrome: a meta-analysis. *Med Sci Monit* 2016;22:736–742.
46. Vetrano IG, Sconfienza LM, Albano D, et al. Recurrence of carpal tunnel syndrome in isolated non-syndromic macrodactyly: DTI examination of a giant median nerve. *Skeletal Radiol* 2019;48:989–993.
47. Liu C, Li HW, Wang L, et al. Optimal parameters and location for diffusion tensor imaging in the diagnosis of carpal tunnel syndrome: a meta-analysis. *Clin Radiol* 2018;73:e11–1058.e19.
48. Klauser AS, Abd Ellah M, Kremser C, et al. Carpal tunnel syndrome assessment with diffusion tensor imaging: Value of fractional anisotropy and apparent diffusion coefficient. *Eur Radiol* 2018;28:1111–1117.

Appendix: Authors

Name	Location	Role	Contribution
Michael D. Pridmore	Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, study concept and design, analysis and interpretation of data, acquisition of data, and statistical analysis.
Gabriella E. Glassman	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, acquisition of data.

(Continued)

Appendix. Continued.

Name	Location	Role	Contribution
Alonda C. Pollins	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, acquisition of data, interpretation of data.
Isaac V. Manzanera Esteve	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Interpretation of data and data analysis.
Galen Perdakis	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript.
Brian C. Drolet	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, study concept and design.
Douglas R. Weikert	Department of Orthopaedics, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, study concept and design.

(Continued)

Appendix. Continued.

Name	Location	Role	Contribution
Mark D. Does	Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, and study supervision.
Wesley P. Thayer	Department of Plastic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, USA	Author	Drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, funding support, and study supervision.
Richard D. Dortch	Department of Neuroimaging Research, Barrow Neurological Institute, Phoenix, Arizona, USA	Author	Drafting/revising the manuscript, study concept and design, interpretation of data, acquisition of data, funding support, and study supervision.