

AWARD NUMBER: W81XWH-17-1-0304

TITLE: Validation of Structural, Molecular, and Functional Imaging Biomarkers in Spinal Cord Injury in Non-Human Primates

PRINCIPAL INVESTIGATOR: John C. Gore

**CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 037232**

REPORT DATE: August 2019

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE August 2019		2. REPORT TYPE Annual		3. DATES COVERED 15 Jul 2018 - 14 JUL 2019	
4. TITLE AND SUBTITLE Validation of Structural, Molecular, and Functional Imaging Biomarkers in Spinal Cord Injury in Non-Human Primates				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-17-1-0304	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) John C. Gore Email: john.gore@Vanderbilt.Edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Vanderbilt University Medical Center Nashville, TN 037232				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel 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 Spinal cord injuries (SCI) impair sensory, autonomic and/or motor functions, and are a leading cause of disability. The process of spontaneous repair of damaged SC is poorly understood because of a lack of appropriate longitudinal tracking methods. Such a method would provide the information needed for a basic understanding of recovery processes and for determining the optimal time window, targets and effectiveness of therapeutic interventions. Combinations of novel, advanced MRI methods can provide unique insights into SCI progression, especially into the functional integrity of grey matter and micro-structural and biochemical changes in white matter. Much previous work in rodent models has limited significance for human injuries, but studies in non-human primates are directly applicable. We propose to use non-invasive multiparametric MRI at high field to assess changes in structural, functional and cellular/molecular properties of SCI over time in a monkey model and determine how these changes predict and correlate with behavioral recovery.					
15. SUBJECT TERMS- NONE LISTED					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
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1. INTRODUCTION:

The research proposed will track longitudinally the spontaneous repair of injured spinal cord in a primate model of cervical spinal cord injury (SCI) using novel, multi-modal MRI methods, and will establish their relationships to gold-standard invasive measures of function and structure in non-human primates. The purpose of the research is to provide the basis for the interpretation of integrated, MRI-based biomarkers for the non-invasive assessment of SCI and repair, which are poorly understood because of a lack of appropriate longitudinal tracking methods. Such information is critical for understanding recovery processes of sensory, autonomic and/or motor functions and for determining the optimal time window, targets, and effectiveness of therapeutic interventions. For the research we will use a combination of advanced MRI methods that provide unique insights into SCI progression, including functional integrity of grey matter and micro-structural and biochemical changes in white matter to assess changes in structural, functional and cellular/molecular properties of SCI over time in a monkey model. We will also determine how these changes correlate with and predict behavioral recovery and histological endpoints for the development of safe and effective biomarkers for monitoring spinal cord injuries and evaluating treatment outcomes.

2. KEYWORDS:

MRI, fMRI, CEST, qMT, DTI, monkey, spinal cord, spinal cord injury, cervical

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Goals: Our SOW identified the following major goals for Y2:

Major Task 1

- (a) Implement DCL injury model in NHPs and acquire MR data following injury: Image NHPs before and at regular intervals following injury, up to 6 months. *Completed*
- (b) Quantify changes in connectivity longitudinally in injured NHPs: Measure connectivity strengths and patterns from fMRI within and across segments at each time point imaged. *Completed*

Milestone 1 for Y2: Completion of two animal cohorts (at 15 and 21 months)
Completion date: May 30, 2019

Major Task 2

- (a) Implement DCL injury model in NHPs and acquire MR structural and molecular data at intervals following injury: Image NHPs before and at regular intervals following injury, up to 6 months. *Completed*

Milestone for Y2: Completion of two animal cohorts (at 15 and 21 months)
Completion date: May 30, 2019.

Major Task 3

- (a) Validate connectivity from resting state MRI by comparisons with (i) electrophysiology and (ii) histology: Compare high resolution resting state fMRI with electrical recordings and injected tracers to verify functional circuits in spinal cord of squirrel monkeys. *75% complete.*
- (b) Perform electrophysiologic recordings from spinal cords of NHPs post SCI at specific time points: Implement surface and microelectrode recordings and measure inter-regional synchrony and coherence. *75% complete*
- (c) Implement DCL injury model in NHPs and quantify changes in behavior longitudinally in injured NHPs; correlate with MRI. Measure behavioral changes on relevant tasks at each time point imaged. *Completed*
- (d) Verify injury locations and nature using histology: Perform post-mortem histological studies with appropriate stains (cytochrome oxidase, Nissl, Myelin, VGluT2) at specific time points, including at the end of 6 months. *75% complete.*

Milestone for Y2: Completion of two animal cohorts (at 15 and 21 months)
(3a, b, d) 75% completed June 10, 2019. Remaining 25% (one subject) to be completed July 18, 2019
(3c) Completion date May 27, 2019

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities

Major activities included (1a) implementing DCL injury to NHPs and acquiring MR data at regular intervals, including pre-lesion (baseline) and post-injury at 1-2 week, 3-month, and 6-month time points; and (1b) assessing changes in connectivity longitudinally in injured NHPs by measuring BOLD signal correlation strengths and patterns from fMRI data within and across segments at each time point imaged. In the same NHP cohorts, (2) MR structural and molecular data were acquired before injury and at regular intervals following injury up to 6 months. Validation of fMRI resting state functional connectivity was achieved by (3) comparing high resolution resting state fMRI data at 9.4T with (i) electrophysiology and (ii) tracers injected for histological analysis to verify functional circuits in the spinal cord of squirrel monkeys. To achieve this, *in vivo* electrophysiologic recordings were acquired from spinal cords of NHPs post-SCI at specific time points (3 and 6 months) via microelectrode recordings to measure inter-regional synchrony and were compared to previously acquired pre-lesion baseline contralateral data. Injury locations and the nature of injury were verified by performing post-mortem histological studies with appropriate stains (cytochrome oxidase, Nissl, Myelin, VGluT2). Anatomical tracers of BDA, CTB and Fluoro-Ruby were injected into functionally identified touch region on the lesion and non-lesion sides to reveal intrinsic intra-spinal connections. Also, behavioral changes were assessed on relevant tasks for each time point imaged before and after injury.

Specifically, behavioral training and assessment on the reach-grasp task (dominant hand) were conducted at least weekly pre- and post-lesion. After determining the handedness of the subject and successful training, pre-lesion MRI scans were acquired. Following the implementation of DCL injury, fMRI data were acquired repeatedly in SCI animals. These data were analyzed to quantify resting state connectivity using seed-based methods to identify circuits and degrees of connectivity between horns, within and across segments. Molecular and structural MRI (CEST, qMT, DTI) data were also collected pre-lesion and post-injury at specific time points in the same animals. Post-processing algorithms developed in the previous year of funding were applied to derive parametric maps for each time point. For the validation of fMRI results by comparison to electrophysiology, we have implemented surface and electrode array recordings of NHP spinal cord at specific time points and measured stimulus-evoked response and LFP coherence at rest for comparison to fMRI resting state functional connectivity data. Verification of injury location and the nature of the injury were assessed using histology with appropriate tracers (GFAP) and stains (cytochrome oxidase, Nissl, Myelin, VGluT2).

2) Specific Objectives

- (1) Determine how the functional integrity of injured spinal cord grey matter repairs over time using functional MRI (fMRI).
- (2) Determine how injured spinal cord white matter repairs over time using structural and molecular MRI.
- (3) Validate our MRI findings with invasive, gold-standard measures. These will include electrophysiology, behavioral measures, and quantitative analyses of histological sections obtained at different time points during the course of recovery.

3) Significant Results

Functional MRI data are critical for quantifying the functional integrity of the nervous system, including the spinal cord. Resting state (RS) fMRI data were collected in the same subjects before and after injury to determine how correlations in BOLD signal, indicative of functional connectivity strengths, between same-slice and between-slice left and right dorsal and ventral horns, change after injury and during recovery. Spinal cord fMRI data were acquired at 9.4T using a 4-shot segmented echo planar imaging sequence in anesthetized, mechanically ventilated squirrel monkeys with a saddle-shaped transmit-receive surface coil positioned over the neck. Image preprocessing included regression of the signals from muscle and cerebral spinal fluid. After injury, the lesion is apparent in the structural magnetization transfer contrast (MTC) image (Figure 1B). Within the injured slice (slice 3) RS functional connectivity significantly decreased between left and right dorsal horns (Figure 1A) at 1-2 weeks post-injury compared to pre-lesion (baseline) correlation measures (Figure 1C). In the injured slice 3, normalization of functional connectivity observed at 3 months was maintained at 6 months post-injury (Figure 1C, 2C).

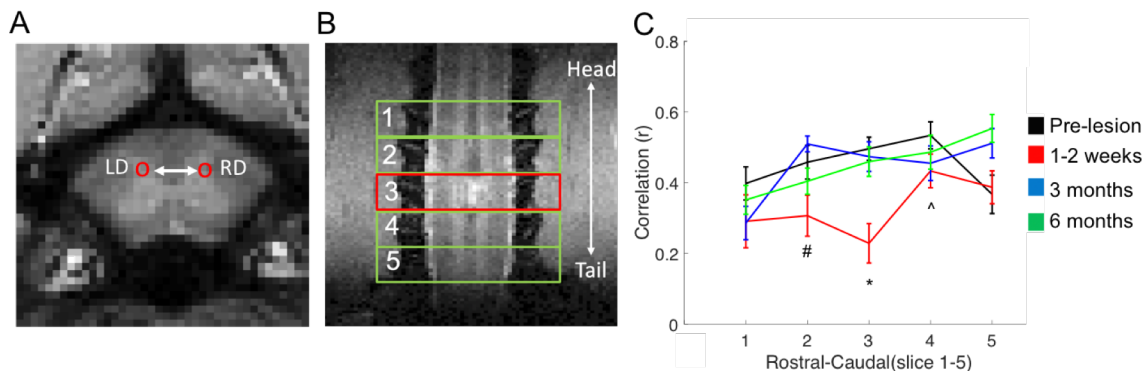


Figure 1. Resting state fMRI correlations within 5 slices before and after cervical spinal cord injury.

(A) Magnetization transfer contrast (MTC) image of one coronal spinal cord slice; left dorsal (LD) and right dorsal (RD) horns indicated by red circles. (B) Sagittal MTC image show the 5 slices analyzed (rostral, top and caudal, bottom); the injury site is at slice 3 (red box). (C) The correlation, r , of BOLD resting state signals between LD-RD horns across 5 slices at pre-lesion (baseline) and post-injury 1-2 weeks (red), 3 months (blue), and 6 months (green). # $p < 0.05$, significant 1-2 weeks and 3 months vs Pre-lesion; * $p < 0.05$, significant 1-2 weeks, 3 month, and 6 months vs Pre-lesion; ^ $p < 0.05$, significant 1-2 weeks vs Pre-lesion (one-way ANOVA).

Focusing on the slices immediately rostral and caudal to the injured slice, left-right dorsal horn BOLD signal correlations decreased within the adjacent slices above (slice 2) and below (slice

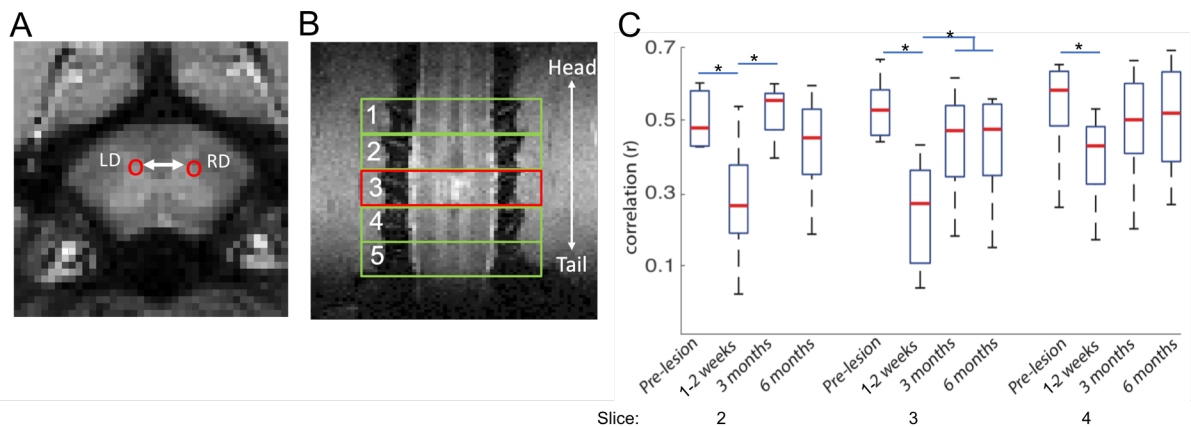


Figure 2. Intraspinal resting-state fMRI correlations at 9.4T in injured and adjacent slices before and after cervical spinal cord injury. (A) Magnetization transfer contrast (MTC) image of a coronal slice indicates left dorsal (LD) and right dorsal (RD) horns (red circles). (B) Sagittal MTC image of the slices (rostral, top and caudal, bottom); the injury site is at slice 3 (red box). (C) Group boxplots of connectivity measures are displayed as Pearson's correlation for fMRI show the median correlation coefficient, r , (red line) at the slice immediately rostral to the lesion (slice 2), the injured slice (slice 3), and caudal slice (4). * $p < 0.05$ (vs. 1-2 weeks, one way ANOVA).

4) the lesion (Figures 1C, 2C), correlations in BOLD signal were lower 1-2 weeks post-injury compared to that at pre-lesion (baseline). At 3 months post-injury, functional connectivity significantly increased in slice 2 from that at 1-2 weeks. In the injured slice 3, this normalization was maintained at 6-months post-injury (Figures 1C, 2C). In slice 4, however, within-slice left-right dorsal horn BOLD signal correlations at 3 and 6 months post-injury were not significantly different from that at 1-2 weeks (Figure 2C).

Figure 3 summarizes functional connectivity strengths for all left and right dorsal and ventral horn combinations across and within all 5 slices. At 1-2 weeks post-injury, the strengths of RS

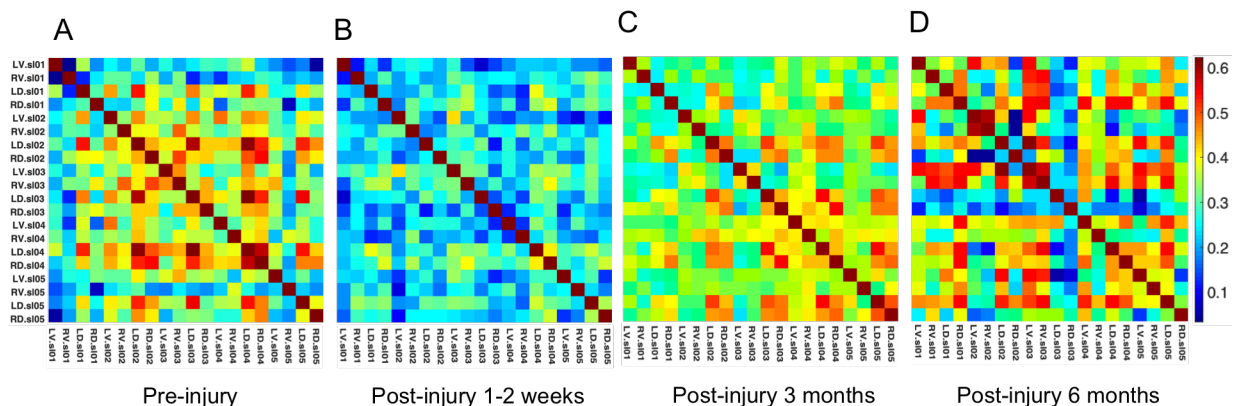


Figure 3. Intra- and inter-slice mean group resting state fMRI correlations across and within 5 slices before and after cervical spinal cord injury. Each matrix depicts mean correlation, r (see colorbar), for region pairs. Regions are left and right ventral and dorsal column for 5 slices at (A) pre-injury, (B) post-injury 1-2 weeks, (C) post-injury 3 months, and (D) post-injury 6 months. Abbreviations: LV=left ventral, RV=right ventral, LD=left dorsal, RD=right dorsal, sl=slice.

functional connectivity, overall, decreased across all segments and regions (Figure 3B) compared to that at baseline pre-lesion (Figure 3A). At 3 months post-injury, there was an overall increase in functional connectivity (Figure 3C) compared to 1-2 weeks post-lesion followed by a further general increase in correlations at 6 months post-injury, except in connections with the left and right dorsal horns to the injured slice (Figure 3D).

Electrophysiology data from spinal cords of NHPs were acquired to measure tactile-evoked responses and LFP coherence between specific regions. First, single epoxylite-coated tungsten microelectrode ($\sim 1\text{M}\Omega$ impedance) with a standard exposed sharp tip ($< 3\mu\text{m}$) were used for mapping the spinal cord. Penetration depths of each microelectrode were recorded and performed at $300\mu\text{m}$ increments. At each interval, the digits of the right and left hands were tapped lightly while receptive field of neurons were characterized. Then, electrophysiological recordings, including tactile stimulus-driven LFP data were acquired. Figure 4 shows data from recordings below the injured site, ipsilateral and contralateral to the lesion. Tactile stimulation to each digit (Figure 4A) elicited LFP responses (Figure 4B). Independent component analysis (ICA), was used to analyze the LFP data. At 3 months post-injury, the change in power decreased significantly on the injured side compared to control (baseline), but not on the contralateral side for digit 2 (Figure 4C). At 6 months post-injury, there was no difference in power compared to baseline control (Figure 4C) indicating normalization, or recovery, of response to tactile input.

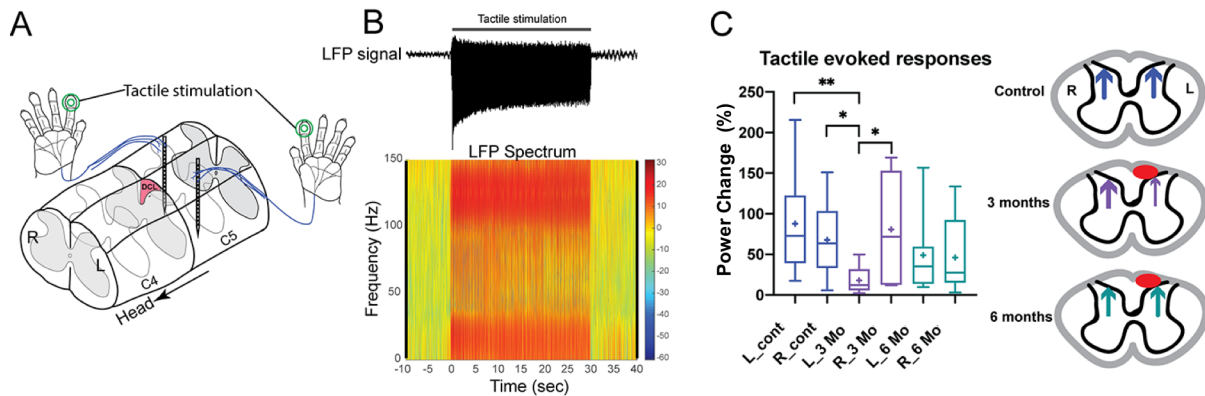


Figure 4. Tactile-evoked LFP responses before and after cervical spinal cord injury. (A) Schematic of spinal cord and digits (digit 2, D2) stimulated. (B) LFP signal (top) and spectrum at baseline and with tactile stimulation. (C) Boxplots show group mean tactile-evoked responses as % power change for left control (L_cont, or pre-lesion), right control (R_cont, or pre-lesion), 3-months post-injury on the left (L_3 Mo) and right (R_3 Mo) sides, 6-month post injury left (L_6 Mo) and right (R_6 Mo). Schematic depicts the regions and strengths (arrow thickness) of power. The median and mean of are represented as horizontal lines and crosshair, respectively, in each boxplot. * $p<0.05$ and ** $p<0.005$, significant.

Figure 5 shows resting state LFP coherence data for the section below the injury site (Figure 5A). LFP coherence at rest between left and right dorsal horns was significantly lower than that at baseline (control) at both 3 and 6 months post-injury (Figure 5B). Other results include LFP coherences for LD-LIGM (contralateral to the injury) and RD-RIGM (ipsilateral to the injury); both significantly increased at 3 months post-injury, but significantly dropped 6 months post-injury (Figure 5C, D).

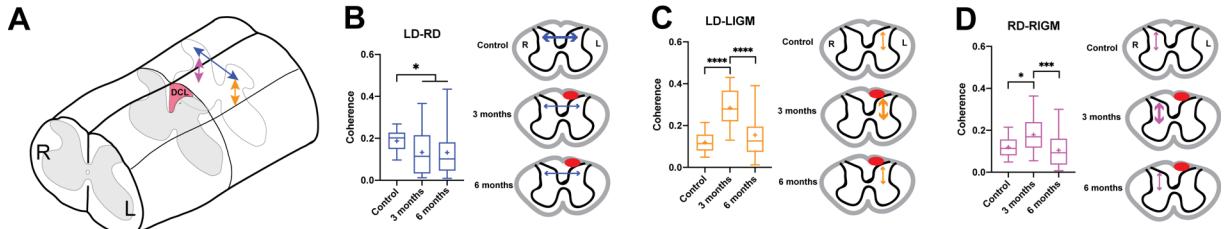


Figure 5. Within-slice LFP coherence data before and after dorsal column lesion (DCL). (A) Schematic of LFP coherence data computed from spinal cord electrophysiology recordings below the injury site (red, DCL). (B) Boxplots show group coherence data for control (pre-lesion), 3-months post-injury and 6-months post-injury for left dorsal-right dorsal (LD-RD) horns with the schematic depicting the spinal regions and coherence strengths, indicated by arrow thickness (thicker is stronger). (C) Boxplots show group coherence data for control (pre-lesion), 3-month and 6-month post injury time points for left dorsal-left intermediate gray matter (LD-LIGM) with the schematic depicting the regions and coherence strengths (arrow thickness). (D) Boxplots show group coherence data for right dorsal-right intermediate gray matter (RD-RIGM) for the control (pre-lesion), 3-month and 6-month post injury time points. The median and mean values are represented by horizontal lines and crosshatches, respectively, for each boxplot. * $p < 0.05$, *** $p < 0.0005$, and **** $p < 0.00005$, significant. The schematic depicts the regions and strengths (arrow thickness) of coherence.

We are performing electrophysiology to validate fMRI resting state functional connectivity results. Here we observe that in the section below the lesion, within slice left dorsal-right dorsal horn LFP coherence (Figure 5B) was higher in the control group (pre-lesion LFP coherence = 0.188) than at 3 and 6 months post injury (LFP coherence=0.132), indicating lack of recovery. Similarly, BOLD fMRI resting state correlations were higher pre-lesion ($r=0.534$) than post-injury ($r=0.455$ at 3 months and $r=0.486$ at 6 months). The differences between the baseline control versus post-injury time points were significant for the LFP coherence data suggesting that this method is more sensitive than fMRI. However, fMRI resting state data so far reflect the same trends as LFP coherence showing reduced functional connectivity within the spine segment below the injury. Concordance between LFP and fMRI metrics will be further analyzed and compared.

Structural and microstructural changes occur after spinal cord injury, including molecular changes and structural (white matter) injury. To measure these changes *in vivo* in monkeys, DTI, CEST, and qMT data were acquired at 9.4T in the DCL injury model at regular intervals following injury.

Diffusion tensor imaging (DTI) was used to quantify changes in white matter pathways in the spinal cord of squirrel monkeys before and after dorsal column injury. For DTI, a respiratory-gated, spin-echo echo-planar imaging protocol was implemented (TR/TE = 3000/35ms; duration of gradient pulse/diffusion time (δ/Δ) = 4/12 ms); amplitude = 19.96 gauss/cm; b-value (1000 s/mm^2); 30 gradient directions; isotropic spatial resolution $0.5 \times 0.5 \times 0.5 \text{ mm}^3$; matrix size $64 \times 64 \times 8$; sampling band width 250 kHz; number of excitations 16). A diffusion tensor was derived for each voxel using the 30 diffusion-weighted acquisitions from different orientations using CAMINO. We calculated fractional anisotropy (FA) and radial diffusivity (RD) (Figure

6B, C) at baseline (pre-lesion) and at 1-2 weeks, 3 months, and 6 months post-injury. In the lesioned slice (slice 3, Figure 6A), there was a significant decrease in FA in the ipsilateral dorsal column at the 1-2 week time point after injury that did not recover by 3 or 6 months post-injury. RD significantly increased 1-2 weeks post-injury compared to pre-lesion values and also remained significantly higher at 3 and 6 months post-injury. Focusing on slice 4 immediately caudal to the injured slice, at 3 months post-injury, FA ipsilateral to the injury was significantly lower than that at pre-lesion. By 6 months post-injury, however, FA recovered in slice 4 (Figure 6B), indicating recovery. RD remained higher in the injured slice (3) at 1-2 weeks, 3 months and 6 months post-lesion compared to RD at pre-lesion (Figure 6C). Unlike FA, no changes in RD were observed in slice 4 below the injury, but in slice 2 rostral to the lesion, RD significantly decreased at 3 and 6 months post-lesion compared to pre-lesion baseline values.

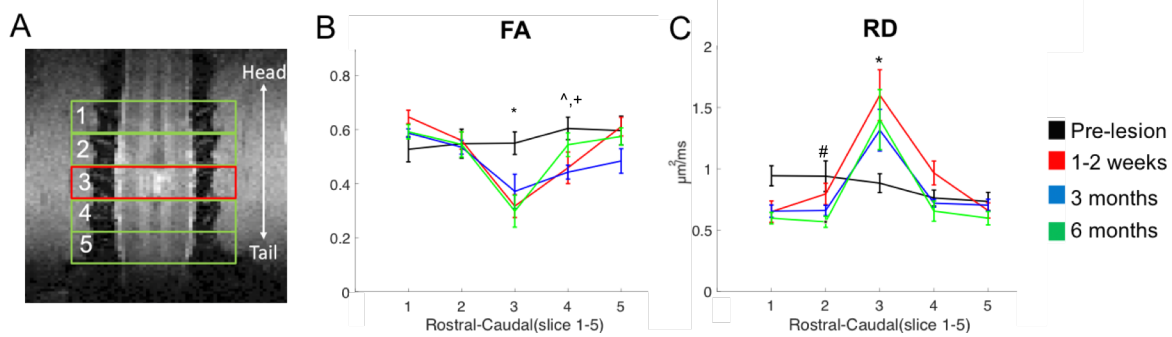


Figure 6. Pre- and post-SCI diffusion parametric changes over the 6-month recovery period in 5 slices. (A) MTC structural image with 5-slice overlay (rostral, top and caudal, bottom); dorsal column lesion injury site is at the center (slice 3, red box). Graphs show (B) FA, fractional anisotropy and (C) RD, radial diffusivity at 4 time points, pre-lesion (black) and post-injury 1-2 weeks (red), 3 months (blue), and 6 months (green) in the 5 spinal cord segments ipsilateral to the injury. * $p < 0.05$, significant (1-2 weeks, 3-months, 6-months vs pre-lesion); # $p < 0.05$, significant (3-months vs pre-lesion); ^ $p < 0.05$, significant (3-months, 6-months vs pre-lesion).

Overall, the functional integrity of injured spinal cord grey matter repairs over time and this can be detected using resting state functional MRI. Injured spinal cord white matter repairs over time and can be assessed by DTI parametrics in the injured section as well as immediately adjacent slices. For DTI, FA and RD over the recovery period demonstrates are also sensitive to injury and recovery. We are comparing our fMRI findings with invasive, gold-standard electrophysiology measures, and show concordance between the two for within slice measures for the section rostral to the injury. BOLD resting state connectivity results validated with electrophysiology suggest that RS fMRI signals acquired at high field directly reflect underlying neuronal activity. We will continue the comparison of resting state fMRI and LFP coherence between other regions as well. Further, behavioral measures and quantitative analyses of histological sections obtained at different time points during the course of recovery are being compared to DTI, CEST, and qMT measures.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project provided training opportunities for a Biomedical Engineering graduate student in fMRI and electrophysiological methodologies and analyses and professional development for a research faculty member through conference presentations.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

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

If this is the final report, state “Nothing to Report.”

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

We will perform studies as outlined in the SOW. This includes completing longitudinal studies in an additional cohort in Y3: (a) pre-lesion behavioral training and assessment has commenced in 4 subjects; (b) pre-lesion MRI/fMRI; (b) implementing the DCL injury model and acquiring MR data at regular intervals following SCI; (c) quantifying changes in functional connectivity longitudinally in injured NHPs in the same cohort; (d) implementing high resolution CEST, qMT, and DTI in the same cohort; (e) validating connectivity from resting state fMRI with electrophysiology, behavior, and histology; and (f) determining the sensitivity and specificity of each MR biomarker, and correlating with electrophysiology, behavior, and histological data.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

None.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or

equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

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).*

Peer-reviewed article: Wu T-L, Yang P-F, Wang F, Shi Z, Mishra A, Wu R, Chen LM, Gore JC. Intrinsic functional architecture of the non-human primate spinal cord derived from fMRI and electrophysiology. *Nature Communications*. 2019; 10: 1416.
(published; yes, federal support acknowledged)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication*

(published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

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.*

Conference Proceedings:

1. Wang F, Zu Z-L, Wu T-L, Yan X, Gore JC, Chen LM. 2019. Rapid and accurate CEST and NOE MRI of injured spinal cord using multi-pool fitting. Proc. Intl. Soc. Mag. Reson. Med. 2876. (published; yes, federal support acknowledged)
2. Wang F, Xu J, Wu T-L, Yang P-F, Byun NE, Chen L-M, Gore JC. 2019. Structural assessment of injured spinal cord using the spherical mean technique and diffusion tensor imaging. Proc. Intl. Soc. Mag. Reson. Med. 2876. (published; yes, federal support acknowledged)
3. Mishra A, Wang F, Chen L-M, Gore JC. 2019. Quantitative assessment of temporal changes in fMRI and DTI parameters in the squirrel monkey spinal cord after injury. Proc. Intl. Soc. Mag. Reson. Med. 3869. (published; yes, federal support acknowledged)

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

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

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Name: Nellie Byun
Project Role: Program Manager
Researcher Identifier (e.g. ORCID ID): 0000-0003-3351-7375
Nearest person month worked: 2.7
Contribution to Project: Dr. Byun has refined and updated animal protocols, collected electrophysiology and behavior data, coordinated activities between the lab and Division of Animal Care (orders, facility transfers), and purchased lab supplies.

Name: Limin Chen
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0001-9662-1925
Nearest person month worked: 1.8
Contribution to Project: Dr. Chen has worked on refining the animal care protocol, participated in the optimization MRI data acquisition protocols and electrophysiology, performed spinal cord surgeries, and worked with the vendor and Division of Animal Care at Vanderbilt on new animal purchases.

Name: John Gore
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-9480-0900
Nearest person month worked: 0.96
Contribution to Project: Dr. Gore has arranged and supervised all of the experiments and analyses performed.

Name: Feng Wang
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-8965-7708
Nearest person month worked: 2.1
Contribution to Project: Dr. Wang has optimized MRI data acquisition protocols, refined the data analysis pipeline, and performed MRI experiments and analyses.

Name: Pai-Feng Yang
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0003-0265-4772
Nearest person month worked: 2.25
Contribution to Project: Dr. Yang has optimized the electrophysiological recording protocols, refined data preprocessing and analysis pipelines, and performed electrophysiology experiments and analyses.

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

Not applicable.

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

Submitted with report.

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

See attached.

ARTICLE

<https://doi.org/10.1038/s41467-019-09485-3>

OPEN

Intrinsic functional architecture of the non-human primate spinal cord derived from fMRI and electrophysiology

Tung-Lin Wu^{1,2}, Pai-Feng Yang^{1,3}, Feng Wang ^{1,3}, Zhaoyue Shi^{1,2}, Arabinda Mishra^{1,3}, Ruiqi Wu¹, Li Min Chen ^{1,3} & John C. Gore^{1,2,3,4}

Resting-state functional MRI (rsfMRI) has recently revealed correlated signals in the spinal cord horns of monkeys and humans. However, the interpretation of these rsfMRI correlations as indicators of functional connectivity in the spinal cord remains unclear. Here, we recorded stimulus-evoked and spontaneous spiking activity and local field potentials (LFPs) from monkey spinal cord in order to validate fMRI measures. We found that both BOLD and electrophysiological signals elicited by tactile stimulation co-localized to the ipsilateral dorsal horn. Temporal profiles of stimulus-evoked BOLD signals covaried with LFP and multiunit spiking in a similar way to those observed in the brain. Functional connectivity of dorsal horns exhibited a U-shaped profile along the dorsal-intermediate-ventral axis. Overall, these results suggest that there is an intrinsic functional architecture within the gray matter of a single spinal segment, and that rsfMRI signals at high field directly reflect this underlying spontaneous neuronal activity.

¹Vanderbilt University Institute of Imaging Science, Nashville, TN 37232, USA. ²Biomedical Engineering, Vanderbilt University, Nashville, TN 37232, USA. ³Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN 37232, USA. ⁴Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37232, USA. These authors jointly supervised this work: Li Min Chen, John C. Gore. Correspondence and requests for materials should be addressed to T.-L.W. (email: tung-lin.wu@vanderbilt.edu)

The identification of patterns of highly correlated, low-frequency fMRI signals in the brain in a resting state has provided a powerful approach to delineating functional architecture and neural circuits noninvasively^{1–5}. Altered resting-state functional connectivity (rsFC) under different pathological conditions suggests that these correlations reflect intrinsic neural processes that are relevant for maintaining normal brain functions⁶. Over the past two decades, there have been thousands of reports of rsfMRI studies of the brain, but only recently has there been an emergence of similar studies in gray matter of the spinal cord^{7–16}. This could be attributed to difficulties that arise in imaging the spinal cord—a small physical size, pronounced physiological noise, and increased effects from susceptibility gradients¹⁷. Nonetheless, recent technical advances have allowed robust detection of strong, rsFC between spinal horns in animal models as well as human subjects^{7,8,10,13,15,16,18}. These observations have led several research groups to hypothesize that, like the brain, spinal cord gray matter exhibits its own intrinsic functional architecture that serves as a fundamental framework for executing and maintaining sensory, motor, and autonomic functions. However, given the complex and indirect coupling of blood-oxygen-level-dependent (BOLD) fMRI signals with electrical activity, validation of their relevance by comparisons to direct measurements of neural activity is critical to allow more precise interpretations of rsfMRI correlations within the spinal cord.

Previous studies have demonstrated a direct link between neural activity and BOLD signals in regions of the brain^{19–24} in stimulation and task conditions. However, direct comparisons of spontaneous electrophysiological activity and rsfMRI signals remain relatively unexplored in the brain^{20,21,25,26}, and even less is known for the spinal cord. In the rat, previous studies have described stimulus- or movement-induced electrophysiological activities in the spinal cord^{27,28}. In particular, Briue et al. measured changes in blood volume and flow using intrinsic optical imaging following electrical stimulation, and subsequently compared optical responses with electrophysiological measurements²⁹. While this study provides complementary insights to the work presented here, no previous study has directly compared and related rsfMRI signals to neural activity within the spinal gray matter in a resting state. The current study aims to address three key questions: (1) what are the intrinsic functional organization features of the spinal gray matter, (2) what is the relationship between changes in stimulus-evoked fMRI signals and the underlying neural electrical activity (local field potentials (LFPs) and multiunit activity (MUA)), and (3) do correlations in rsfMRI signal fluctuations (indicators of resting state FC) reflect the underlying synchronous variations in spontaneous neural electrophysiological activity within the spinal gray matter from the same regions.

In this study, we combined measurements of LFPs and MUA in non-human primates (NHPs) to validate findings from stimulus-driven and resting-state fMRI signal changes. Strong agreements were found between the two modalities for both the spatial patterns of metrics of neural activities and their inter-regional correlations: tactile stimulation evoked activations at ipsilateral horns in both fMRI and electrical recordings, while resting-state correlations between regions showed parallel relationships. This study identifies an intrinsic functional architecture relating dorsal–intermediate–ventral areas, and validates the interpretation of rsfMRI studies of the spinal cord. We propose that this will provide a foundation for using fMRI for assessing and monitoring alterations in spinal cord circuits that occur with injury and other pathologies.

Results

Comparable tactile responses between the two modalities. To determine the relationship between spinal fMRI signals and neural electrophysiological activity during the processing of innocuous tactile inputs, we recorded and compared the temporal variations of BOLD, LFP, and MUA signals during the presentation of 8-Hz vibration stimulations of a single distal pad in a block design experiment. Figure 1e illustrates the quality of our sub-millimeter fMRI acquisitions, and Fig. 1g shows an example of the average BOLD activation map from one monkey obtained at 9.4 T. Figure 1f shows that 8-Hz innocuous tactile stimulation of a single digit elicited the strongest BOLD signal changes in the ipsilateral dorsal horn, with a peak value of $0.76 \pm 0.06\%$. Figure 2 shows representative stimulus-driven LFP and multiunit spike activity recorded from the ipsilateral horn that receives inputs from the stimulated digits, which also demonstrate strong responses to stimulation. LFP voltage changes followed the stimulus time course (yellow shades in Fig. 2b, e) and frequency peaks are apparent at harmonics of 8 Hz in the LFP power spectra (Fig. 2c, f). This observation was consistent among all four monkeys that underwent electrophysiological testing. Group-normalized LFP power changes showed digit-selective responses to the tactile stimulus (compare the four different color curves in Fig. 2a, d). Similarly, in line with the LFP observation, spike rate histograms as well as peri-event raster plots of spike density in the ipsilateral horn also showed the largest responses to the single digit that was stimulated (Fig. 2h, k). Group-normalized spike rates obtained from four different recording sites (left D3, right D3, left D5, and right D5) are presented in Fig. 2g, j. Figure 3 presents group-averaged BOLD signal time courses and neural signal (LFP and MUA) responses to tactile stimulation overlaid on the same plot. Overall, significant and correlated changes in all three measures were observed in the ipsilateral dorsal horn of the homeotopically appropriate cervical segment, a finding that is congruent with fMRI results here and in previously reported studies^{30,31}.

Local dorsal spatial resting-state correlation profiles. Previous studies in NHP spinal cords revealed a strong functional connectivity between dorsal–dorsal and dorsal–ventral horns. Taking advantage of the high signal-to-noise ratio (SNR) and the resolution of high-field rsfMRI data, we plotted the local correlation profiles of the signals from a dorsal horn seed region along the dorsal–intermediate–ventral axis. Small regions of interest (ROIs) were drawn from the dorsal toward the ventral horn on one side of the spinal cord (Fig. 4a). In over half of the observations (58% of the runs), we found that correlations demonstrated a U-shaped pattern in which correlation strengths decreased monotonically from a high value to a low value at the level of the intermediate gray matter, and then increased as the ventral region was approached (Fig. 4b, c and Supplementary Figure 1). In the remaining runs, the correlations decreased continuously. This suggests the existence of three distinct subdivisions (dorsal, intermediate, and ventral), and allowed the intermediate GM seed ROIs to be more confidently defined for subsequent comparisons with electrophysiology (see details in the Methods section). This local correlation profile was supported at the group level after aligning the defined intermediate GM ROIs shown in Fig. 4c. The temporal SNR (tSNR) of the voxels examined did not show systematic changes as a function of distance from the seed (Fig. 4d), suggesting that the correlation profile was not driven by varying noise levels across three zones.

Validation of rsfMRI connectivity of the spinal horns. Inter-horn resting-state connectivity patterns observed in rsfMRI were

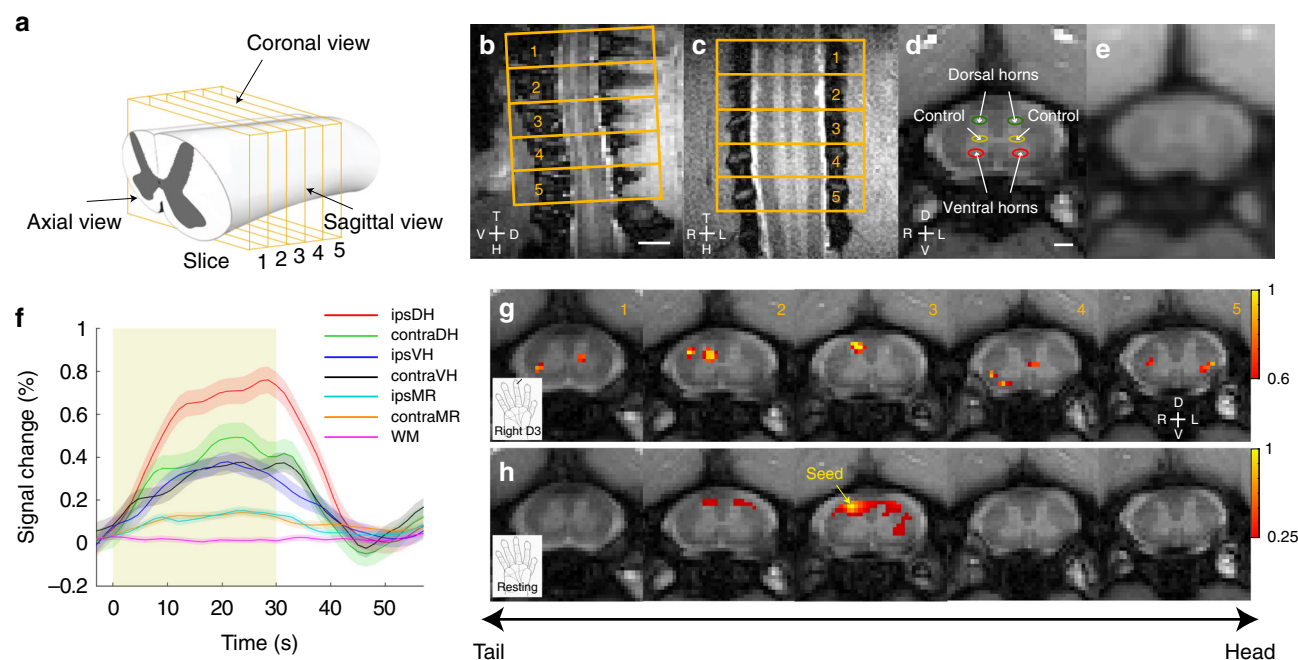


Fig. 1 Stimulus-driven and resting-state fMRI in non-human primates at 9.4 T. **a** Schematic diagram (modified from Hollis et al.⁵⁵) of imaging planes of the spinal cord. **b** Sagittal and **c** coronal views of the spinal cord in magnetization transfer contrast (MTC) images. Scale bar represents 3 mm. **d** MTC and **e** BOLD-sensitive axial images. Scale bar represents 1 mm. Red and green circles indicate dorsal and ventral horns, respectively. Yellow circles present intermediate gray matter of the spinal cord used as controls for later quantifications. **f** Group-averaged ($N = 7$ monkeys) BOLD signal changes in the four horns—dorsal horn (DH) and ventral horn (VH)—of the spinal cord and middle/intermediate gray matter regions (MR) that are ipsilateral (ipsi) and contralateral (contra) to the stimulus, as well as white matter (WM) control region. Shaded error bars represent standard error of mean. **g** Multirun activation map to D3 tactile stimulation thresholded at 0.6 of normalized percentage signal, with a peak value of 1. **h** Multirun resting-state connectivity patterns (thresholded at $r > 0.25$) of a seed from one representative monkey. D dorsal, V ventral, H head, T tail

compared with LFP connectivity patterns derived by analyzing broadband coherences between signals from different elements in each linear array. Connectivity measures between different specific pairs of ROIs were compared: dorsal–dorsal (within-slice), dorsal–intermediate gray matter, and dorsal–dorsal (across slice) (Fig. 5a). At the group level ($N = 12$ and 4 monkeys for fMRI and LFP, respectively), within-slice dorsal–dorsal functional connectivity was observed to be stronger (mean BOLD $r = 0.49$ and LFP coherence = 0.19) than that in dorsal–intermediate gray matter (averaged mean between left and right BOLD $r = 0.40$ and LFP coherence = 0.12) for both fMRI and LFP (Fig. 5b, c). Moreover, LFP coherence was found to be statistically significant at depths (from the dorsal surface) up to 1.5 mm between contralateral regions at precisely the same depth (Supplementary Figure 2A, B). For the four monkeys that underwent electrophysiology and fMRI recordings, linear regression between electrophysiology coherence and rsfMRI correlation revealed an r -value of 0.5079 ($p = 0.0058$) (Supplementary Figure 2C). The within-slice resting-state connectivity was robust between horns, but across-slice correlation strengths were found to be consistently lower (mean BOLD $r = 0.26$ and LFP coherence = 0.04). Trends of connectivity measures for different ROI pairs (Fig. 5b, c) were also highly similar between the two modalities and Bonferroni–Holm corrected Mann–Whitney tests between different ROI selections support this conclusion.

Discussion

We found in this study that within-slice (representing one spinal segment) dorsal–dorsal horn resting-state functional connectivity is significantly greater than connectivity values measured between dorsal horns and reference regions, identified here as intermediate gray matter regions in the cord, for both fMRI and LFP.

Comparisons of how connectivity values varied between the three ROIs (dorsal–dorsal within-slice, dorsal–intermediate–GM, and dorsal–dorsal across the slice) between the two modalities revealed that they are highly similar. Previous spinal rsfMRI studies have also reported the existence of strongly correlated bilateral sensory networks in anesthetized rodents and NHPs^{13,15}, and awake humans. In humans, an early report used independent component analysis, a data-driven approach, to separate the spinal cord into dorsal and ventral networks¹⁶. We used an ROI-based technique that showed robust BOLD correlations between dorsal–dorsal and ventral–ventral horns, with no significant group-level correlations between gray and white matter regions⁷. The reproducibility of these findings was further quantified and confirmed^{7,9}, while more recent studies have explored the variable patterns of the functional network¹² and the influences of physiological noise on measurements of functional connectivity¹¹. In animal studies performed at 9.4 T, similar conclusions were obtained: strong dorsal–dorsal and ventral–ventral connections were found, compared with control white matter regions^{13,15}. The biophysical basis of these findings to date remains unclear for the spinal cord. Moreover, whether correlated fMRI signals between spinal ROIs reflect similar neural processes as cortical ROIs also remains largely unknown. Our results using direct electrophysiological measures help fill this knowledge gap, although our findings here focused only on the relationships of BOLD and LFPs in the sensory dorsal horns. Overall, our results demonstrate that rsfMRI can be a reliable surrogate biomarker for evaluating neural circuits in the spinal cord, and the strength of BOLD correlations reflects neural functional connectivity, as indicated by spontaneous LFP signals.

Communication between spinal segments is critical for executing spinal cord functions, but intersegment (cross-slice)

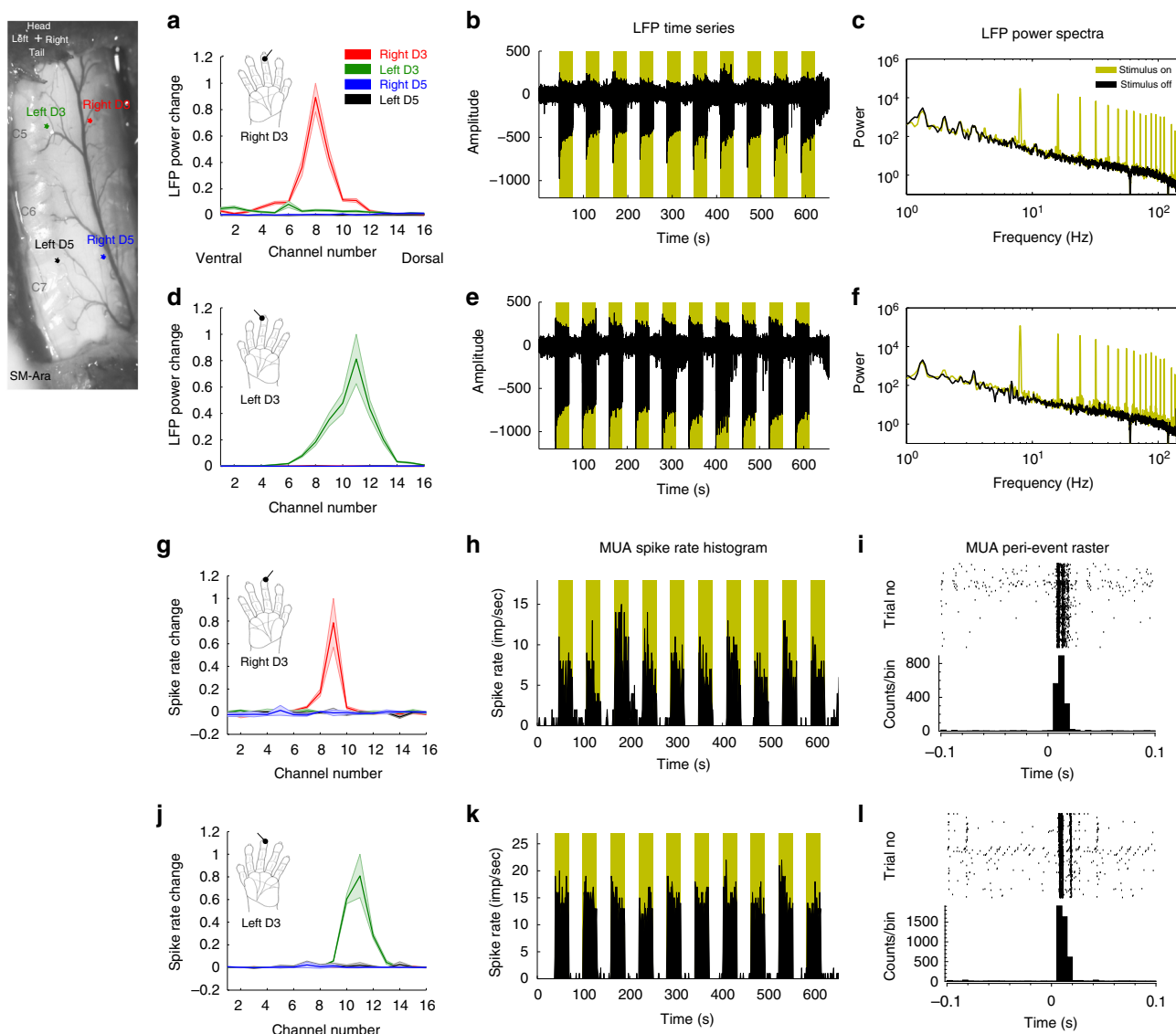


Fig. 2 Stimulus-driven LFP and spike activity responses in one representative monkey, SM-Ara. **a, d** Normalized averaged ($n = 3$ runs) LFP power changes computed from Welch's power spectra, and **(g, j)** normalized spike rate ($n = 3$ runs) changes between 30 s stimulus-on and -off in all four electrodes under right-D3 and left-D3 innocuous 8-Hz tactile stimulus conditions, respectively. Shaded error bars represent standard error of mean. **b, e** LFP time series from the channel that presents the greatest power change and its respective **(c, f)** Welch's power spectra. Dark yellow shaded regions and plots represent stimulus-on periods. **h, k** Spike rate histograms at the channels corresponding to the largest changes and their corresponding **(i, l)** raster plots (top row) and peri-event histograms (bottom row). Spike activity was processed for only two of the four monkeys (SM-Ara and SM-Bus)

resting-state connectivity has been little studied. In humans, Kong et al. reported no correlations between resting-state signals between different segmental levels using an independent component analysis¹⁶. More recently, Liu et al. showed significant functional connectivity across slices and vertebral levels, although most of them were located within one segment distance¹². In our previous investigations in NHPs, we also found a strong functional connectivity between the same horn (e.g., dorsal to dorsal) on two adjacent segments, but with resting-state correlation values decreasing from $\sim r = 0.85$ to $\sim r = 0.4$ and $\sim r = 0.3$ when moving one and two slices away, respectively¹³. A similar phenomenon was observed in rodents where connectivity decreased significantly as distances along the spine between ROIs increased¹⁵. With direct neuronal recordings, we aimed to answer the question of whether this observation is a possible artifact of image processing or image acquisition applied on a slice-by-slice basis, or because correlations truly decrease with increasing

separation along the cord. Our rsfMRI and electrophysiology results both indicate the latter. The diminished tactile responses two segments away from a stimulated digit's receptive field are also consistent with this finding. The segmental organization of the spinal cord permits somatotopic encoding of peripheral information, and perhaps intrinsic circuits are also organized in such a fashion. As expected, LFP measurements showed greater statistical differences between across- and within-slice connectivity ($p < 0.00005$ Bonferroni-Holm corrected Mann-Whitney test), and parallel those from fMRI.

Logothetis et al. showed, with simultaneous recordings of BOLD and neural signals in the visual cortex, that a spatially localized increase in BOLD contrast corresponds directly to an increase in local neural activity²². In addition, LFP responses were observed to be statistically greater and more maintained throughout a stimulus than MUA²². Our stimulus-driven results unequivocally showed that increased fMRI signal

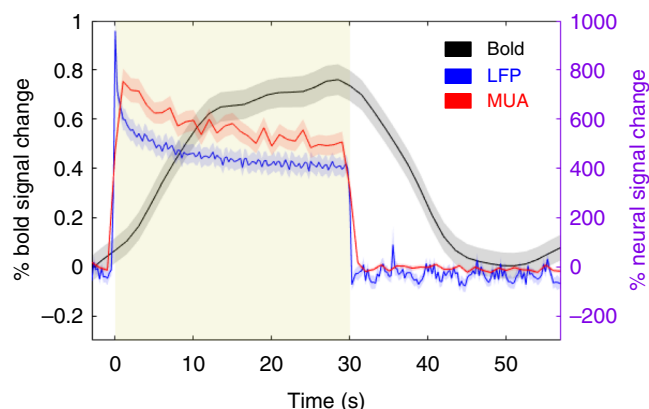


Fig. 3 Responses to innocuous tactile stimulation of digits in the spinal cord. Overlay of averaged time courses of fMRI (black), LFP (blue), and MUA (red) responses in the ipsilateral dorsal horn. Shaded yellow region and error bars represent stimulus-on period and standard error of mean, respectively. LFP signals are represented with computed r.m.s. (bin size = 0.25 s, no overlap, $N = 4$ monkeys, $n = 40$ runs), while MUA is presented as spike rates (bin size = 1 s, no overlap, $N = 2$ monkeys, $n = 24$ runs). Percentage signal changes for BOLD and neural signal were computed relative to the pre-stimulus period; BOLD and electrophysiological signals are plotted against the y-scale on the left (black) and right (purple), respectively. It is also important to note that signals from the two modalities were not acquired simultaneously

changes correspond to increased neural electrophysiological activity. Robust LFP responses were observed in all four monkeys, and both MUA and LFP responses were maintained throughout the 30-s duration stimuli, a result similar to our observations in the cortical area 3b of squirrel monkey³². Moreover, our observations within the spinal cord again support the notion that regions which are engaged collectively in the same function, such as processing an external stimulus (e.g., tactile input), demonstrate a strong resting-state functional connectivity between them^{6,13,33–35}. In particular, ipsilateral dorsal horns of the spinal cord have been shown to be most responsive to tactile stimulations^{31,36}, as further verified with both fMRI and electrophysiology in this study. In humans, innocuous tactile stimulation produces responses localized in the ipsilateral dorsal gray matter as well as in areas around the gracile and cuneate nuclei, with the overall activation pattern in line with the dorsal–medial lemniscus pathway³⁶. Consistent with this observation, we also previously found tactile-evoked responses predominantly toward deeper regions of the ipsilateral dorsal horn in monkeys at 9.4 T³¹. Interestingly, responses in contralateral dorsal gray matter were also detected in both studies, and this could be attributed to a descending projection effect and/or commissural connections between bilateral dorsal horns. Using electrode positions that responded most robustly to tactile stimulation as seeds, these channels demonstrated stronger connectivity patterns with each other compared with others. Overall, these studies extend the notion that baseline functional connectivity is an organizational feature of the central nervous system and occurs in the spinal cord, and replicates patterns similar to stimulus-evoked activation foci. The observation that regions working together in tasks exhibit a strong functional connectivity at rest appears to be a universal organizational principle within the central nervous system.

While spinal electrophysiology has previously been performed in other animal models, the functional architecture and neural circuits of the central nervous system in NHPs share much greater similarities with those in humans. Previous reports have

made use of NHPs to compare and understand the underlying biophysical basis of BOLD signals, but these were restricted to different cortices of the brain³⁷. In this study, we have used NHPs to perform direct validation of MRI findings in the spinal cord using multichannel microelectrodes. That being said, some rsfMRI spinal connectivity differences between NHPs and humans in previously published reports are also present. In humans, within the hemi-cord, dorsal–ventral connectivity remains speculative and shows weaker correlations compared with bilateral dorsal and ventral horn connectivity^{8,9,18}, although unilateral resting-state dorsal components have also been identified using independent component analysis¹⁶. In NHPs, however, ipsilateral dorsal–ventral connectivity appears to be significant when compared with controls¹³, and this could be attributed to differences in the functional organization of the spine between species if there is a greater use of coordinated bilateral behaviors in animals. Despite some differences present, the study of NHPs provides a crucial link between invasive animal data and human studies^{33,38}.

As described in previous publications^{17,30,39,40}, imaging the cervical spinal cord poses several challenges. Using an optimized MRI sequence along with modified image processing steps¹³, we have reduced these confounding effects and developed a sensitive protocol for the detection of resting-state networks in the cervical spinal cord. Electrophysiological recordings of the spinal cord also pose their own challenges. For example, there is a trade-off between the number of recording sites (four were chosen in our study) and the density of electrode contacts for sampling.

Another limitation of this study is that the ventral horns were not fully sampled due to the design of the recording electrodes, which included a dead space between the tip of the electrode and the first recording contact of the linear array. Ventral–ventral connectivity has been shown to be robust and reproducible in both humans and animals^{7,15,41}. In support of this finding, a number of studies have also reported the presence of commissural interneuron connections between ventral horns^{42,43}. Resting-state fMRI correlations identified the corresponding U-shaped profile of connectivity that should be observable by LFP recordings using different electrode designs. In addition, the observation of hemi-cord dorsal–ventral connectivity has been less consistent. Eippert et al. reported that ROI selection appears to influence the observed dorsal–ventral connectivity, which suggests that this may be a partial volume effect that causes mixing of time courses due to the proximity of dorsal and ventral horns⁴⁴. Given the involvement of sensorimotor systems in mediating reflexes in the spinal cord, it remains unclear why dorsal–ventral connections have not been reliably detectable. One possible explanation is that spinal cord neurons do not exhibit their full network of connections at rest⁴⁴. Nevertheless, to fully examine the functional relationship between dorsal and ventral horns, sampling of both regions simultaneously by electrophysiology will permit more comprehensive comparisons with fMRI, but requires further developments in electrode configurations.

Achieving a high spatial resolution with adequate SNR in fMRI is critical for minimizing partial volume effects as well as for characterizing functional connectivity at fine scale. In this study, high SNR and resolution were achieved by imaging a stable preparation at 9.4 T, by acquiring images with a multi-shot gradient-echo sequence (a technique used in Barry et al., 2016, 2014)^{7,45}, and by reducing TE (a technique used in Zhao et al., 2009, 2008)^{46,47}. Further potential improvements may be obtained using better RF coils (e.g., cryogenic coils) and deploying more advanced shimming methods.

Finally, a possible confounding factor is the influence of anesthesia in the measurement of functional connectivity for both modalities. An increase in anesthesia level has been shown to

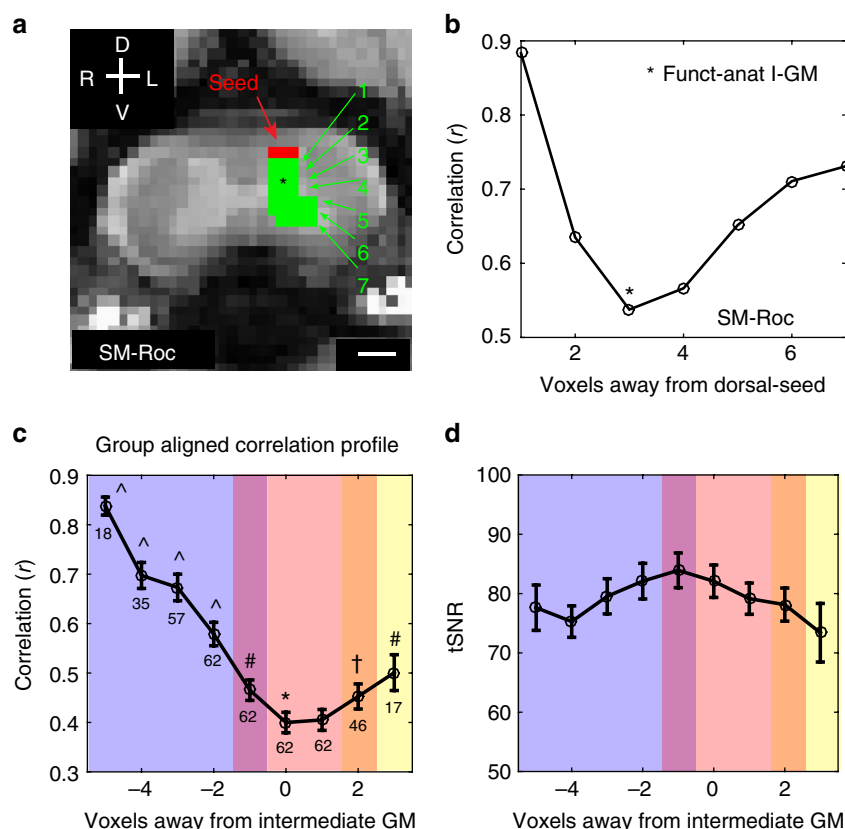


Fig. 4 Local dorsal-to-ventral correlation profile. **a** ROIs defined in one representative monkey on one side of the spinal cord. Mean time series of the dorsal region (red voxels) was used as a seed to compute mean Pearson's cross-correlations with voxels from each depth (green voxels); depth numbers are indicated by the green numbers. Scale bar represents 1 mm. **b** An example of an observed U-shaped profile from SM-Roc. Asterisk (*) represents defined intermediate GM (I-GM) seeds based on functional and anatomical features; greatest difference of connectivity value to the dorsal horn seed. **c** Group-averaged aligned correlation profile based on each run's anatomically functionally defined seed of the intermediate GM region for both left and right sides of the spinal cord. Error bars represent standard error of mean. The number of observations for each distance are indicated below each scatter circle. Data points representing four voxels away from I-GM were removed as they contain only five observations. Blue, red, and yellow shades represent estimated dorsal, intermediate, and ventral boundaries defined based on two-sided Mann-Whitney test comparisons (corrected for false discovery rate) relative to the defined I-GM seed; $^{\wedge}p < 0.000005$; $^{\#}p < 0.05$; $^{\dagger}p < 0.1$. Shades of overlaid colors—purple and orange—represent an estimated overlap of dorsal-I-GM and ventral-I-GM, respectively. Supplementary Tables 1 and 2 provide uncorrected and corrected p -values for Mann-Whitney Tests and r -values, respectively. **d** Group-averaged tSNR at different depths of the spinal cord used to compute (**c**)

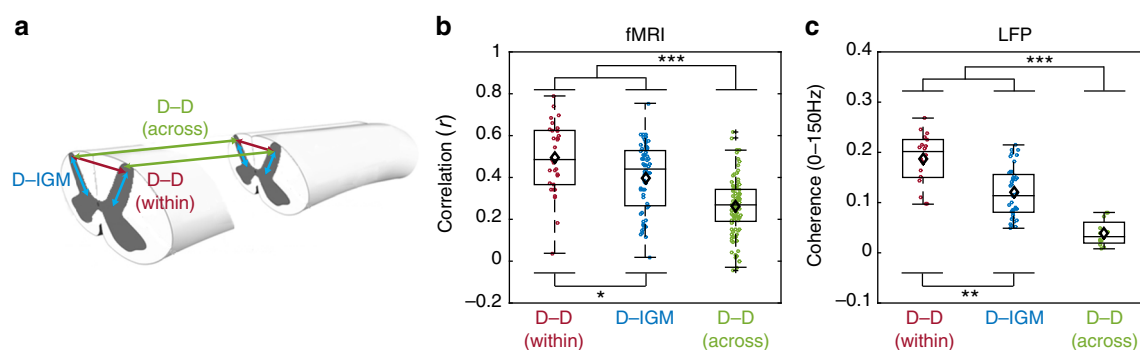


Fig. 5 Comparison between resting-state fMRI and LFP connectivities. **a** Schematic diagram of correlation and coherence computed in the recordings of the spinal cord. **b, c** Group averaged within and across segments dorsal to dorsal (D-D) as well as dorsal to intermediate GM (D-IGM) connectivity was computed. Group boxplots of connectivity measures are displayed as Pearson's correlation for fMRI and averaged coherences for LFP. Each boxplot contains fMRI observations from 12 monkeys (including those that underwent electrophysiology) across 15 functional studies, and electrophysiology observations from four monkeys in panels **b** and **c**, respectively. Ipsilateral dorsal-to-intermediate GM from the left and right hemi-cord was concatenated. The median and mean are represented as horizontal lines and diamonds, respectively, in each boxplot. Scatter circles on each boxplot represent individual observations. Using mean connectivity values from the three ROI pairs from both modalities (**b, c**), Pearson's correlation value was computed to be 0.9982. $^*p < 0.05$, $^{**}p < 0.0005$, and $^{***}p < 0.00005$ Bonferroni-Holm corrected two-sided Mann-Whitney test

reduce cortical functional connectivity in NHPs^{48,49}, while others have indicated the possibility of neurovascular decoupling at high anesthesia dosages (see a review in Masamoto and Kanno, 2012)⁵⁰. That being said, studies have also demonstrated that anesthesia has a weaker influence in early sensory cortical regions⁵¹, and thus, this effect is most likely reduced in the downstream spinal cord. In our experiment, the isoflurane was maintained at <1%, which is lower than the range where significant neuronal connectivity activity drops and becomes unstable in the brain (1.75% isoflurane reported in ref. ⁴⁸). In fact, a recent study found that neurovascular coupling in the spinal cord remains unaltered at 1.2% isoflurane in an experiment with decerebrated rats undergoing electrical stimulation. Moreover, simultaneous recordings of local evoked field potentials and fMRI in brain studies have shown that signals from the two modalities are still coupled under anesthesia^{22,26,52–54}. With this information, we believe that anesthesia is unlikely a major confounding factor to the trends observed in this study.

Methods

Animal preparation. Adult male squirrel monkeys (*Saimiri boliviensis*) were used in this study. Four animals (SM-Ara, SM-Leg, SM-Gua, and SM-Bus) underwent laminectomy and subsequent electrophysiological recordings (Fig. 6). For both MRI and electrophysiological experiments, animals were first sedated with ketamine hydrochloride (10 mg/kg)/atropine sulfate (0.05 mg/kg, i.m.) and maintained with isoflurane anesthesia (0.5–1.2%) delivered in a 70:30 N₂O/O₂ mixture. After intubation, animals were mechanically ventilated, monitored, and infused intravenously with 2.5% dextrose in saline solution (2–3 ml/h/kg) in order to prevent dehydration. Animals' vital signals, including peripheral oxygen saturation and heart rate, EKG, end-tidal CO₂, and respiratory pattern, were continuously monitored. With a circulating water blanket, temperatures of the animals were also kept between 37.5 and 38.5 °C. Animals were subsequently placed in a custom-designed MR cradle and onto a stereotaxic frame for MRI and electrophysiological experiments, respectively. Extra care and efforts were put into ensuring that the animal's neck was secured and straight while ear bars were also used to minimize further motions. While the isoflurane level fluctuated over a range depending on each animal's physiological condition, anesthesia level was generally maintained at 0.7–0.8% isoflurane during functional data acquisitions. For both MRI and electrophysiological experiments, animal monitoring and preparation were performed in almost identical manners. All animal procedures were in compliance and approved by the Institutional Animal Care and Use Committee of Vanderbilt University.

MRI data acquisition and analysis. MRI acquisitions were obtained on a 9.4 T Varian magnet with a saddle-shaped transmit–receive surface coil positioned over the neck. High-resolution anatomic axial images were obtained using magnetization transfer contrast (TR/TE = 220/3.24 ms, 0.25 × 0.25 × 3 mm³), while BOLD images were acquired using a fast gradient-echo sequence (TR/TE = 46.9/6.50 ms, matrix size = 64 × 64, field of view = 32 × 32 mm², resolution = 0.5 × 0.5 × 3 mm³, and flip angle ~15°, ~3 s/volume). Resting-state (300 volumes) and stimulus-driven (8-Hz innocuous tactile, 30 s on/off, seven epochs) acquisitions were both obtained in 12 and 7 animals, respectively. Previously acquired spinal fMRI datasets with a higher temporal resolution (TR = 24.0 ms, ~1.5 s/volume) on healthy monkeys were included in group analyses^{13,31}. We included data acquired with slightly different imaging parameters in order to increase the number of runs and animals in the group average, thereby increasing the reliability of estimates of functional connectivity strengths for comparison with electrophysiological measurements. The different timings are not expected to affect correlations. Specifically, resting-state fMRI included animals that underwent electrophysiology, while stimulus-evoked fMRI contained four monkeys from our previous study³¹ and the first three animals that underwent electrophysiology (SM-Ara, SM-Leg, and SM-Gal).

Previous studies evaluated preprocessing methods for de-noising spinal fMRI data^{17,30}. In this study, ROI data analyses were similar to those used in our previous publications^{13,31}. Specifically, functional image volumes were first aligned using a 2D rigid body motion correction algorithm based on maximization of mutual information, with three motion parameters estimated (two translations and one rotation) slice by slice. BOLD images were then upsampled to 0.25 × 0.25 × 3 mm³ by linear interpolation to match a set of anatomical images. Manual alignment was then performed between the functional images and the corresponding structural images. This step was performed for each slice and for all the runs in each animal. For each slice, “nuisance” signals were derived from muscle and cerebrospinal fluid voxels using principal component analyses. The first three to five signal components that accounted for at least 70% of the cumulative signal variance, along with motion correction parameters, were used as signal regressors in a general linear model to mitigate their contributions to each BOLD time series. fMRI time series were then band-pass filtered with a pass band of 0.01–0.1 Hz using a Chebyshev Type II filter. Runs in which the tSNR in gray

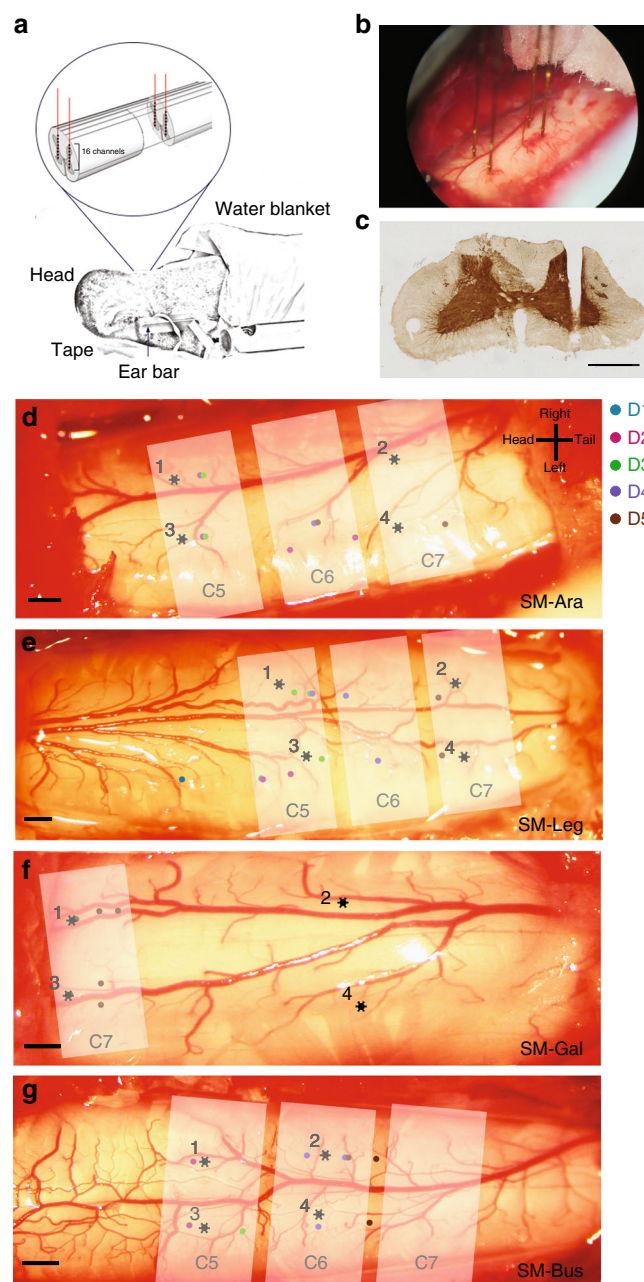


Fig. 6 Electrophysiological recording setup, recording sites, and maps of different digit segments for the four squirrel monkeys (SMs). **a** Schematic diagram of the spinal cord inserted with four electrodes, each with 16 channels that are 150 μ m apart. **b** Sample photograph of the four inserted electrodes in SM-Ara. **c** Cytochrome oxidase stain used to verify electrode penetrations of the spinal cord by identifying the electrolytic lesion. Exposed spinal cord with mapped digit regions (color dots) and recording sites (black asterisks) for **(d)** SM-Ara, **(e)** SM-Lego, **(f)** SM-Gua, and **(g)** SM-Bus. Scale bars represent 1 mm. Shaded white regions are estimated segments of the spinal cord

matter was <50 were removed from the study. In order to eliminate possible partial volume effects due to smoothing introduced by linear interpolation from functional to anatomical resolutions, a cluster threshold of 2 was applied to prevent spurious single-voxel correlations (Fig. 1g, h). Spinal cord masks, excluding voxels outside GM and WM, were also applied. Finally, ROIs were carefully defined before ROI-based analysis.

Electrophysiological mapping and recording. The average fMRI activation maps obtained from seven monkeys were used as guidance for microelectrode

penetrations into the exposed spinal cord. The electrophysiological experiments in this study were divided into two phases: a mapping phase, during which the response areas of the spinal cord were mapped, and subsequently a recording phase, using microelectrode arrays. During the mapping phase, single epoxylite-coated tungsten microelectrodes (~1-M Ω impedance) with standard exposed sharp tips (<3 μ m) were used. The responses of each microelectrode were recorded at different penetration depths separated by 300- μ m increments. At each interval, the hand digits of the animal were tapped lightly to allow the receptive fields of neurons to be established. The magnitudes of responses were also evaluated qualitatively by listening to an audio representation of spike activity and the viewing of action potential traces. Segments of the spinal cord were then identified based on known receptive field properties as well as the somatotopic organization of digits. During the recording phase, using the maps of digit responses, four linear microelectrode arrays (Microprobes Blackrock, 16 channels, 150- μ m separation between contacts) covering various depths were then carefully inserted into targeted recording sites (Fig. 6a, b). LFP broadband voltage signals and spiking activity exceeding a threshold were recorded using a Multi-channel Cerebus Neural Signal Processor system (Black Rock, Millard county, Utah), which received digitized signals at a sampling rate of 30 kHz, and a local reference. LFP signals were recorded continuously with a lowered sampling rate of 500 Hz and low pass filtered at 250 Hz. MUA was also recorded by capturing timestamps of spikes evoked using the same system. Spike processing employed a band-pass filter of 250–5000 Hz, while a global spike detection threshold was set to –4 times the root-mean-squared energy. For stimulation of each digit, 2–3 trials were obtained using the same 30-s on-/off-paradigm as for fMRI with 10 epochs, while resting-state signals were recorded for a duration of 15 min.

Stimulus-driven LFP and MUA data analysis. LFP signals sampled at 500 Hz were notch-filtered at 60 and 120 Hz before a band-pass filter (pass band between 1 and 150 Hz) was applied. Stimulus-driven (Fig. 2) LFP data were then separated into stimulus-on and stimulus-off periods, which were identified as 20 s before and after onset times, respectively. Welch power spectra were subsequently computed (with Hamming window apodization, window length = 10 s, overlap = 50 ms). The percent power changes between stimulus-on and -off conditions at the first five harmonics of 8 Hz were calculated and normalized to the upper bound of the greatest signal. LFP recordings were analyzed for all four monkeys, while spike activity was analyzed for only two monkeys (SM-Ara and SM-Bus), because our initial electrophysiology experiments were optimized specifically for collecting LFP responses. For example, thresholds set for spike event recordings were too high and were not ideal, resulting in minimal spike events being recorded in the initial experiments. Spike rate histograms (bin size = 1 s) and peri-event rasters (bin size = 0.005 s) were computed using NeuroExplorer software. At the group level, time series at channels with the greatest LFP responses were averaged across animals, runs, and epochs, and root-mean-square (r.m.s.) values were computed in successive 0.25-s windows, while averaged spike rate histograms (bin size = 1 s) were also calculated similarly. These plots were converted to percentage changes in spike rate or LFP signals at each timepoint during stimulus-on relative to the averaged spike rate during pre-stimulus baseline signals (3 s before the onset of a stimulus), and subsequently overlaid on the extracted fMRI BOLD activation time course (Fig. 3).

Stimulus presentation protocol. In order to ensure a stable contact between the probe and the animals' fingers, small pegs were glued to the fingernails of the hand, which were firmly embedded into plasticine. This setup leaves the glabrous surfaces of each digit exposed for innocuous vibrotactile stimulation by a 2-mm-diameter rounded plastic probe that is attached to a piezoelectric device driven by a Grass S48 square wave stimulator. During periods with no stimulation, the probe was in light contact with each digit. For fMRI experiments, tactile indentations (0.34-mm vertical displacement) of the probe were presented for 30 s on followed by 30 s off (= one epoch) at 8 Hz with a pulse duration of 20 ms. In total, seven epochs were typically presented within one imaging fMRI run. The same tactile stimulus protocol was used for electrophysiological recordings with 10 epochs. Multiple runs (2–3 runs) were collected for each digit stimulation and stimulus-on and -off timestamps were recorded.

Resting-state LFP data analysis. Resting-state LFPs were further de-noised by notch-filtering the first five harmonics of the respiration frequency. Dorsal seeds at each shank of the electrode that were responsive to tactile stimulation were identified as channels with the greatest LFP responses. Recordings from electrodes 2 and 4 of SM-Bus were excluded due to residual contamination from respiratory and cardiac motion. Intermediate gray matter, which serves as a reference tissue, was subsequently identified as the channel closest to 1 mm away from the dorsal seed on the same shank. This measurement was made based on the corresponding high-resolution MRI axial images. Resting-state magnitude-squared coherences, which are a function of the power and cross-power spectral densities of two signals, were then computed between dorsal horns within and across segments and used to quantify the functional connectivity between the regions. Dorsal-to-intermediate gray matter coherences were also measured within each segment. Similarly, functional connectivity with the contralateral side of the spinal cord was computed using coherences between channels distant from the dorsal seeds on each

shank. Because penetration depths of each monkey were slightly different, measurements that contained data from only one monkey were not considered for group analyses.

ROI selection of intermediate GM. ROI voxels in the intermediate GM control regions were selected based on both anatomical features and functional patterns relative to the ipsilateral dorsal horn. Specifically, small ROIs (2–5 voxels wide (i.e., across the cord) $\times$ 1 voxel deep (i.e., in a dorsal–ventral direction)) were drawn from dorsal to ventral horn on one side of the spinal cord (Fig. 4a). The dorsal horn (red voxels) was subsequently selected as the seed. Mean correlations between resting-state time series were then computed for all the ROIs as a function of depth (green). The correlations demonstrated a U-shaped pattern in which correlation strengths decreased monotonically from a high value to a low value approaching the intermediate region, and then increased again as the ventral region was approached (Fig. 4b). We then functionally and anatomically defined the most appropriate intermediate GM seeds as those voxels at the depth that showed the greatest difference in correlation from the dorsal seed (typically 4–5 voxels deeper). Given the unclear anatomic boundaries of the intermediate GM region, we believe that this allowed a more precise identification of a control intermediate region. Our results showed that a method of defining the intermediate GM region through anatomical features alone may encompass a region contaminated by functional signals from the dorsal or ventral horns, and thus may misplace the most appropriate location of the control region. For sessions in which not all runs showed this profile, the intermediate GM region was defined using results from those runs which were U-shaped. Otherwise, they were identified anatomically as voxels at the same depth as the central canal of the spinal cord. This was repeated for all runs for both left and right sides of the spinal cord. This pattern was supported at the group level with aligned correlation profiles at the defined intermediate-GM ROI shown in Fig. 4c.

ROI-based and statistical analyses. An ROI-based correlation analysis was used to assess functional connectivity between different ROIs. This method was selected because the ROIs could be drawn based on stimulus-driven data as well as previous fMRI studies in humans and animals. We have previously shown that sensory evoked responses are located in the dorsal and ventral horns³¹, so here we evaluate how specific ROIs in dorsal and ventral horns are interconnected in a resting state. Dorsal ROIs were manually selected (2–3 voxels) using co-registered high-resolution MTC-weighted images that showed a high contrast between the white and gray matter butterfly. This was done for both the left and right side of the spinal cord for each monkey. Pearson's correlation coefficients were subsequently computed between different ROI pairs of interest. Our quantitative connectivity measurements between ROIs were similar to those described in our previous publications^{7,8,13,15}. Briefly, mean time series of m individual voxel time series of an ROI (either dorsal or ventral horn of the spinal cord) were correlated with n time series of another ROI. Subsequently, the average of this correlation vector was selected as the metric of functional connectivity between the two ROIs. This was performed for different pairs of ROIs of interest within (middle slice, slice 3 of Fig. 1) and across slices (slices 2, 3, and 4 of Fig. 1). Statistical tests of significance were performed between correlation values using nonparametric two-sided Mann–Whitney Wilcoxon tests. Bonferroni–Holm corrections were performed, and $p < 0.05$ was considered to be statistically significant.

Histology. At the conclusion of invasive electrophysiological recording sessions, animals were given a lethal dose of anesthetic (sodium pentobarbital) before being perfused transcardially with phosphate-buffered 0.9% saline, then 2–4% paraformaldehyde in phosphate buffer, and last 10% sucrose in phosphate buffer. For one of the monkeys (SM-Gua), an electrolytic lesion was made by passing a current (10 μ A) via an electrode in one of the recording sites in the spinal cord (Fig. 6c). This lesion was made to further confirm our sampling region of the spinal cord. The spinal cord was subsequently extracted before sections of the spinal cord were stained for cytochrome oxidase to locate electrode penetration.

Data availability

A reporting summary for this article is available as a Supplementary Information file. The source data underlying Figs. 1F, 2, 3, 4, and 5 are provided as Source Data files at <https://figshare.com/s/830745f89f8d2377867>. Data and codes supporting the conclusions presented here will be made available following reasonable requests to authors.

Received: 7 October 2018 Accepted: 5 March 2019

Published online: 29 March 2019

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Acknowledgements

This study is supported by the NIH grant NS092961 and the DOD grant SC160154. The authors gratefully acknowledge Fuxue Xin, George Wilson III, Dr. Qing Liu, and Chaohui Tang for their assistance with data collection. We also thank Dr. Chia-Chi Liao for advice on spinal cord recordings, and Dr. Robert L. Barry, Dr. Baxter Rogers, and Benjamin Conrad for their advice on spinal functional data analysis.

Author contributions

J.C.G. and L.M.C. designed and supervised the research; T.-L.W., P.-F.Y., Z.S., R.W., F.W., and L.M.C. performed the animal studies; T.-L.W., P.-F.Y., and A.M. contributed new analytical tools and analyzed the data; and T.-L.W., L.M.C., and J.C.G. wrote the paper.

Additional information

Supplementary Information accompanies this paper at <https://doi.org/10.1038/s41467-019-09485-3>.

Competing interests: The authors declare no competing interests.

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Journal peer review information: *Nature Communications* thanks Jonathan Brook, Patrick Stroman and Wen-Ju Pan for their contribution to the peer review of this work. Peer reviewer reports are available.

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Rapid and Accurate CEST and NOE MRI of Injured Spinal Cord Using Multi-Pool Fitting

Feng Wang^{1,2}, Zhong-Liang Zu^{1,2}, Tung-Lin Wu^{1,3}, Xinqiang Yan^{1,2}, John C. Gore^{1,2,3}, and Li Min Chen^{1,2}

¹Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, TN, United States, ²Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, United States, ³Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, United States

Synopsis

This study aims to evaluate the accuracy of estimates of the amplitudes of specific CEST and NOE effects in Z-spectra derived from multi-pool fitting using down sampled data, and their sensitivity for assessing injury-associated changes in spinal cords of squirrel monkeys. An inverse subtraction analysis corrected for changes in the apparent water longitudinal relaxation rate R_{1obs} (AREX_{mf}) was used to quantify regional spectra around lesion sites. The results provide an optimized rapid, sensitive, and accurate protocol for deriving both NOE and CEST effects in spinal cord imaging at high field.

Purpose

Chemical exchange saturation transfer (CEST) and relayed nuclear Overhauser enhancement (NOE) effects in Z-spectra provide information on metabolites, mobile proteins and membrane lipids that may characterize spinal cords after injury. Accurate and specific quantifications of CEST and NOE signals are important for practical applications¹ but complete, high resolution Z-spectra require multiple acquisitions. Multi-pool fitting of the various resonances can separate different CEST and NOE effects from direct water saturation (DS) and semi-solid MT effects. It is also critical to reduce effects of R_1 variations to CEST and NOE measurements. This study aims to evaluate the accuracy and sensitivity of multi-pool fitting from a reduced set of measurements obtained by down sampling the number of RF offsets used for CEST imaging of injured spinal cord, and to assess injury-associated spatiotemporal changes in CEST and NOE measures in cervical spinal cords of non-human primates (NHP).

Methods

MRI scans were recorded of anesthetized squirrel monkeys (N = 9) at 9.4T, before and after a unilateral dorsal column sectioning (~2 mm) of the cervical spinal cord (Fig. 1A). CEST images were acquired using a 5s continuous wave (CW) saturation of amplitude 1.0 μ T (51 RF offsets equally spaced from -5 ppm to 5 ppm) followed by a spin-echo echo-planar-imaging acquisition (TR/TE = 7500/18 ms, 2 shots, resolution = 0.5x0.5 mm², slice thickness = 1 mm). The amplitudes of CEST and NOE effects from multiple proton pools were quantified using a six-pool Lorentzian fitting of each Z-spectrum². The down-sampled data with different reduced selections of RF offsets were analyzed and compared. The correlation between results derived from down-sampled data (about 1/2 and 1/3 reductions) and data with all 51 RF offsets (P51e) for each specific CEST and NOE effect was evaluated using linear regression, with the assumption that fitting results based on all sampling data with 51 RF offsets provide the best results. The inverse subtraction analysis with correction of apparent water longitudinal relaxation rates R_{1obs} (AREX_{mf}) and multi-pool MTR_{mf} were calculated for comparison¹. The significance of differences in regional pool amplitudes was evaluated using Student's *t*-tests.

Results

Figure 1B shows representative fitting results for normal and abnormal tissues with complete sampling data (P51e) delineating the peak amplitude and width of each CEST or NOE resonance in cervical spinal cord of NHP after injury. The peak amplitude and width can be used to guide the selection of sampling rate in each RF offset domain of the six pools for down sampling. The influences of number of RF offsets and gaps in different RF offset domains on fitting and mapping were evaluated, and the twofold reduction in number of RF offsets (P25e and P26s) yielded comparable accuracy with P51e (Fig. 2). Appropriate interpolation improved the accuracy of the modeling results. All the maps from multi-pool fitting detected unilateral changes at the site of injury, especially in the dorsal pathway and dorsal horns on the side of injury for this particular subject (Fig. 3). Significant decreases in NOE(-3.5) and NOE(-1.6)

Figures

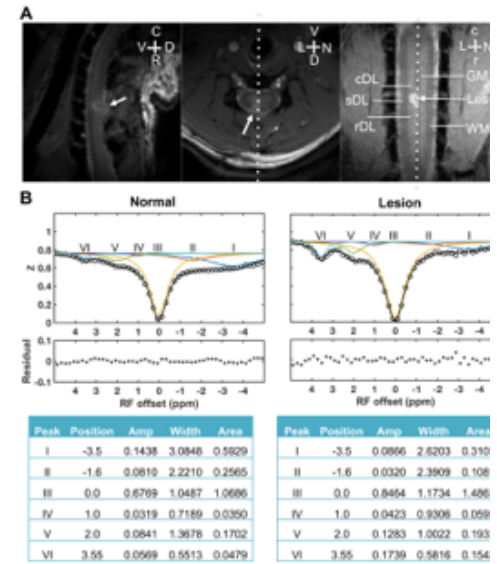


Figure 1. Characteristic features of Z-spectra of tissues in the injured spinal cord of squirrel monkey. (A) Anatomical images with magnetization transfer contrast showing the unilateral dorsal column lesion in the cervical spinal cord. Arrows indicate the lesion site. r: rostral; c: caudal; V: ventral; D: dorsal; L: lesion side; N: non-lesion side. (B) 6-pool Lorentzian band fitting of normal and abnormal tissues of injured spinal cord. Normal: from normal tissues on the non-lesion side of spinal cord; Lesion: from abnormal tissues on the lesion side of spinal cord.

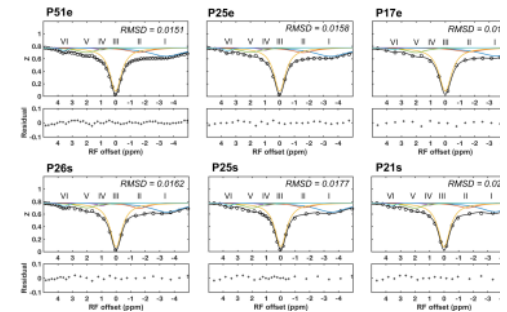


Figure 2. Impact of down sampling on the multi-pool fitting results. P51e, P25e, and P17e: equally spaced CEST data with 51, 25 and 17 RF offsets respectively (0.2, 0.4 and 0.6 ppm intervals respectively); P26s, P25s, and P21s: CEST data with selected 26, 25 and 21 RF offsets respectively

and increases in CEST(1.0), CEST(2.0), and CEST(3.5) were detected at the lesion site (Fig. 3). The map derived from twofold down-sampled data P26sl51 showed quite similar spatial distribution as that obtained using P51e at each RF offset (Fig. 3). The results from P26sl51 of 9 subjects were highly correlated to those using P51e. Across 9 subjects, although both CEST(2.0) and CEST(1.0) increased at the lesion site, they were not as significant as CEST(3.5), compared to relative measures of the tissues on the non-lesion side after the spinal cord injury (Fig. 4). Representative R_1 -corrected $AREX_{mfit}$ of cyst regions also showed significant increases at 3.5 and 2.0 ppm and decreases at -1.6 and -3.5 ppm (Fig. 5). Some abnormal tissues around the lesion site also exhibited significant changes in $AREX_{mfit}$, especially at 3.5 and -3.5 ppm RF offsets. Spatiotemporal variations in the measurements across individual subjects are still under investigation.

Conclusion

These results support the selection of RF offsets and down sampling in CEST imaging of injured spinal cord as a means to reduce total imaging time and/or permit additional signal averaging. $AREX_{mfit}$ provides multiple specific effects without R_1 contamination. The current approach allows a fast (~13 mins) and robust approach to characterize the tissue property changes caused by injury at 9.4T.

Acknowledgements

We thank Mrs. Chaohui Tang and Mr. Fuxue Xin of the Vanderbilt University Institute of Imaging Science for their assistance in animal preparation and care in MRI data collection. This study is supported by DOD grant W81XWH-17-1-0304, and NIH grants NS092961 and NS078680.

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(different intervals for different RF offset ranges). RMSD: root-mean-square deviation of model fitting.

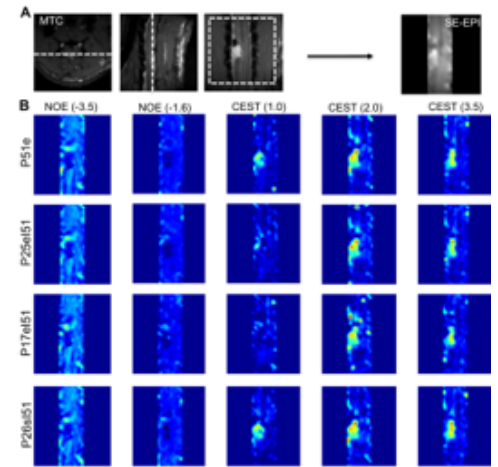


Figure 3. CEST and NOE maps derived from model fitting using selected down-sampled CEST data. (A) Coronal slice for CEST imaging was based on transverse and sagittal slices, and it went through dorsal column. (B) P51e, P25sl51, and P17sl51: equally spaced CEST data with 51, 25 and 17 RF offsets respectively (0.2, 0.4 and 0.6 ppm intervals respectively); P26sl51: CEST data with selected 26 RF offsets (different interval for different RF offset range). Spline interpolation (0.2 ppm interval) was applied to the above down-sampled data before model fitting. Data is from one subject 10 weeks after lesion.

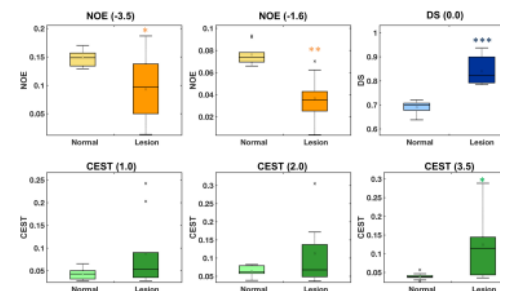


Figure 4. Comparison of regional pool amplitudes of injured spinal cords across subjects (N=9). Boxplots of regional pool amplitudes of normal tissues on the control side and abnormal tissues on the lesion side of spinal cords are compared. The circles and middle lines represent mean

and median values respectively. The crosses represent outliers. $*p<0.05$, $**p<1\times10^{-2}$, $***p<1\times10^{-3}$ versus the values of normal tissues on the control side.

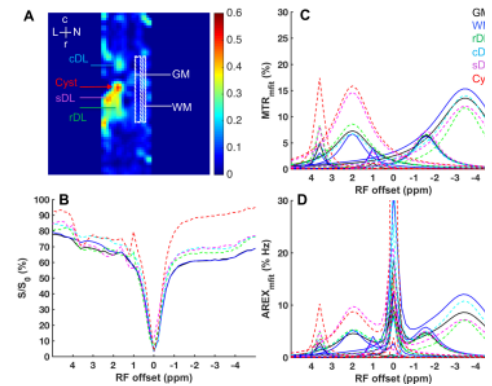


Figure 5. Representative regional AREX_{mfit} results. (A) Six regions of interest in the injured spinal cord, shown in the CEST (2.0 ppm) map. GM: gray matter; WM: white matter; rDL, cDL, and sDL: rostral, caudal and lateral tissues next to lesion site; Cyst: formed at lesion site and confirmed by histology sections. r: rostral; c: caudal; L: lesion side; N: non-lesion side. (B) Regional Z-spectra. (C) Regional multi-pool MTR_{mfit} without R₁ correction. (D) Regional multi-pool AREX_{mfit} results. Regional T_{1obs} is 1.925, 1.585, 1.976, 1.600, 2.001, and 2.366 sec for GM, WM, rDL, cDL, sDL and Cyst, respectively.

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Structural Assessment of Injured Spinal Cord Using the Spherical Mean Technique and Diffusion Tensor Imaging

Feng Wang^{1,2}, Junzhong Xu^{1,2}, Tung-Lin Wu^{1,3}, Pai-Feng Yang^{1,2}, Nellie E. Byun¹, Li Min Chen^{1,2}, and John C. Gore^{1,2,3}

¹Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, TN, United States, ²Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, United States, ³Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, United States

Synopsis

High-resolution spherical mean technique (SMT) MRI may provide specific diffusion measures to detect and characterize structural connectivity before and after spinal cord injury and during repair. This study aims to systematically evaluate the SMT-derived diffusion parameters and compare them to conventional DTI-derived diffusion parameters in assessing injury-associated changes in spinal cords of squirrel monkeys. The main goal is to optimize a rapid and robust high-resolution SMT imaging protocol for spinal cord of monkeys at high field. In addition, the sensitivity of SMT and DTI measures in detecting axonal damage in the dorsal pathway has been evaluated.

Purpose

This study aims to systematically evaluate the feasibility and precision of diffusion measurements based on the spherical mean technique (SMT) for assessing microstructural properties of the spinal cords of non-human primates. This study also aims to characterize changes in different diffusion measures in individual subjects, both regionally and longitudinally, in order to evaluate their sensitivity for detecting tissue structural change after cervical spinal cord injury (SCI).

Methods

MRI scans were performed on isoflurane-anesthetized squirrel monkeys at 9.4T, before and after a unilateral section (~2 mm) of the dorsal column white matter tract (Fig. 1).¹ The spin-echo diffusion sequence used an echo planar imaging readout (TR/TE = 3000/33 ms, 4 shots, resolution = 0.333x0.333 mm², slice thickness = 3 mm, 5 slices). For diffusion imaging, three b-shells were acquired with b values at 750, 1000, and 2000 s/mm² respectively, sampling 30 directions. Three non-diffusion weighted scans were acquired, one at the beginning of each b-value shell acquisition. SMT data were acquired from the cervical spinal cords of seven normal and five spinal cord injured monkeys. SMT derived the apparent axonal volume fraction V_{ax} and intrinsic axonal diffusivity D_{ax} .² The extra-axonal transverse diffusivity D_{ex} was estimated as a function of V_{ax} and D_{ax} . The influences of variations in the acquisition scheme on SMT were evaluated. Conventional DTI parameters including the fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) were also quantified for comparison. Regional correlations between diffusion measures were calculated using the Pearson correlation function. The significance of group regional differences was evaluated using Student's *t*-tests. $P < 0.05$ was considered statistically significant.

Results

DTI and SMT measurements delineated normal white matter (WM) and gray matter (GM) in the cervical spinal cord of monkey (Fig. 2) with good contrast. Regions of WM (LPN, VPN and DPN) showed significantly higher V_{ax} and D_{ax} than GM (VHN and DHN), and conventional DTI parameters FA and AD showed similar regional trends. The measured D_{ex} of GM was slightly higher than that of WM, while RD of GM was significantly higher than that of WM. The SMT-derived measures V_{ax} and D_{ax} were strongly correlated with each other, and they also showed high correlation with DTI measures FA and AD. Although DTI measures showed higher precision across slices than SMT measures at the same SNR level, it is of note that a twofold reduction in acquisition yielded comparable accuracy with SMT. All the DTI and SMT measures detected unilateral changes at the site of injury, especially in the dorsal pathway (Figs. 3-4). A significant decrease in V_{ax} was also observed in rostral and caudal regions next to the lesion site ($p < 0.05$), while FA decreased. Although both D_{ax} and D_{ex} increased at the lesion site, D_{ex} showed higher unilateral contrast than D_{ax} compared to the non-lesion control side twelve days after

Figures

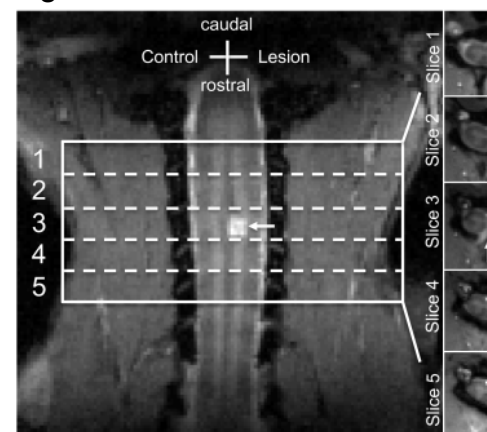


Figure 1. MTC images showing unilateral dorsal column lesion in cervical spinal cord of monkey. The white box on the horizontal image indicates the field of view and selected slices (thickness at 3 mm) for diffusion imaging over cervical spinal cord. Arrows indicate the lesion site. Images were obtained from a subject 4 weeks post-lesion.

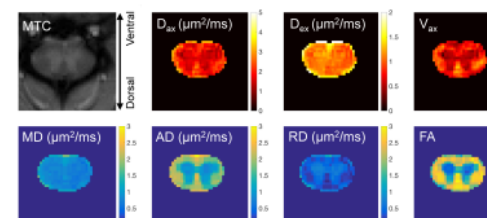


Figure 2. Comparison of characteristic diffusion maps from SMT (top row) and DTI (bottom row).

spinal cord injury. In this representative injured subject, the rostral region to the lesion showed a more severe damage than the caudal region (Fig. 3). This is also seen in the statistical results across subjects (Fig. 5). It is notable that D_{ax} and AD increased at the lesion/cyst but decreased in the tissues of the dorsal pathway rostral or caudal to the lesion site (Figs. 4-5). SMT-derived V_{ax} was sensitive for detecting axonal damage and recovery in the dorsal column pathway after unilateral dorsal column lesion, comparable to FA (Figs. 3-5). Longitudinally, evident recovery from injury is revealed by changes of these diffusion parameters.

Conclusion

Diffusion imaging can provide an efficient and sensitive means to detect and characterize axonal damage after injury and monitor its recovery over time. SMT modelling provides a robust and alternative method to DTI analysis for characterization of the damage of spinal cord after injury, independent of axonal orientation.

Acknowledgements

We thank Mrs. Chaohui Tang and Mr. Fuxue Xin of the Vanderbilt University Institute of Imaging Science for their assistance in animal preparation and care in MRI data collection. We also thank Mr. Ken Wilkens and Dr. Xinqiang Yan for customizing coils for cervical spinal cord imaging. This study is supported by DOD grant W81XWH-17-1-0304, and NIH grant NS092961.

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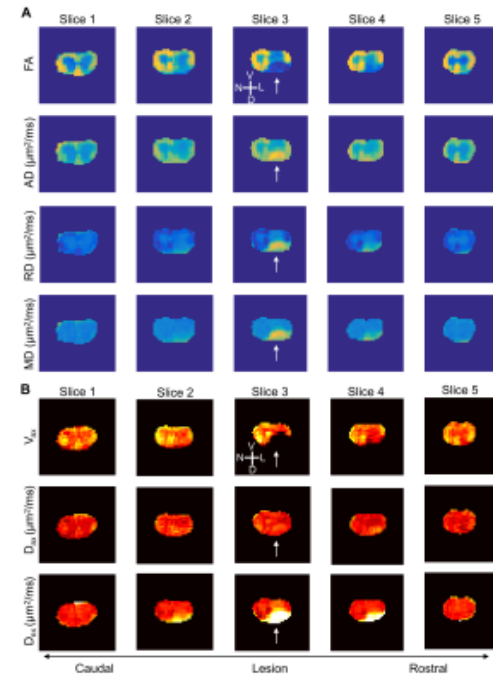


Figure 3. Spatial information revealed by diffusion parameters after unilateral dorsal column lesion of cervical spinal cord. (A) DTI parameters. (B) SMT parameters. Arrow indicates the lesion site. V: ventral; D: dorsal; N: non-lesion side; L: lesion side. Data is from one subject 2 weeks after lesion.

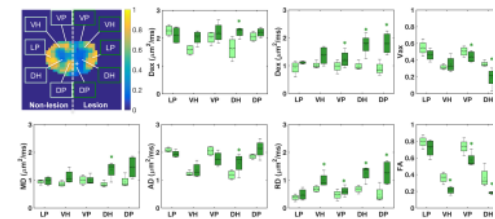


Figure 4. Regional comparison of diffusion parameters at lesion site in cervical spinal cord. Regions of interest are shown in FA map. The plus symbol (+) in the map indicates the lesion site. In the boxplots, middle lines indicate median and circles indicate mean values across subjects. Parameters for the non-lesion side are in light green, and those for lesion side are in dark green. VP: ventral pathway; VH: ventral horn; LP: lateral pathway; DH: dorsal horn; DP: dorsal pathway. $*p < 0.05$ versus the respective regional values on the non-lesion side.

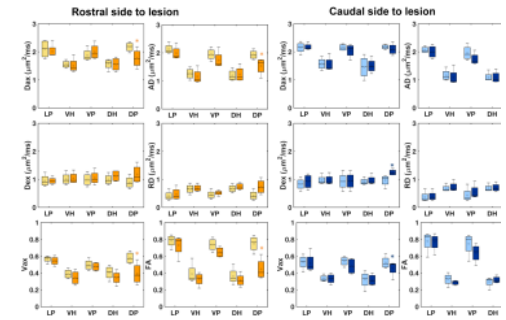


Figure 5. Regional comparison of diffusion parameters in tissues rostral and caudal to the lesion of cervical spinal cord. In the boxplots, parameters for the non-lesion side are in light color, and those for lesion side are in dark color. Middle lines indicate median and circles indicate mean values across subjects. VP: ventral pathway; VH: ventral horn; LP: lateral pathway; DH: dorsal horn; DP: dorsal pathway. * $p < 0.05$ versus the respective regional values on the non-lesion side.

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Quantitative Assessment of Temporal Changes in fMRI and DTI Parameters in the Squirrel Monkey Spinal Cord after Injury

Arabinda Mishra¹, Feng Wang¹, Li Min Chen¹, and John C Gore¹

¹Radiology, Vanderbilt University, Nashville, TN, United States

Synopsis

Previous studies have reported changes in resting state functional connectivity between horns in spinal cord following injury. In this work we quantified changes in connectivity and diffusion parameters at identical spinal segments in squirrel monkey cervical spinal cords acquired in concurrent sessions to compare the temporal variations of functional and structural changes during recovery after spinal cord injury (SCI). The degree of axonal disruption and demyelination measured using fractional anisotropy and radial diffusivity at two stages of recovery were compared in various white matter tracts.

Introduction

Correlated fluctuation of low frequency BOLD signals in a resting state between horns of gray matter have been detected in spinal cords in humans, rats and monkeys [1,2,3]. This resting state functional connectivity (rsFC) is weakened following injury but recovers over time [3]. Diffusion tensor imaging (DTI) has been used to measure structural changes along the white matter tracks in the spinal cord and to assess axonal disruption and demyelination [4, 5]. In this work we analyzed the similarity and differences between DTI and rsFC measures along the C4-C8 segments in axial images acquired in the same sessions in squirrel monkeys at 9.4T before and after a targeted unilateral sectioning of the dorsal white matter tract. The temporal changes of DTI parameters of fractional anisotropy (FA) and radial diffusivity (RD) in the injured dorsal track were analyzed and related to changes in rsFC between two pairs of ROIs in grey matter horns, at two time points during recovery after the injury along the cord cross spinal segments.

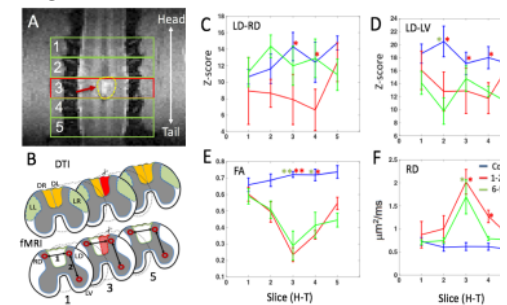
Material and Method

MRI images were acquired with an Agilent 9.4T scanner using a saddle-shaped transmit-receive surface coil of anaesthetized squirrel monkeys. Four animals participated in the study whose dorsal column tract spinal cords were later lesioned on one side at C5 level to acquire post SCI data. A fast gradient echo sequence (flip angle = $\sim 18^\circ$, TR = 46.88ms, TE: 6.5ms, 3s per volume) was used to collect resting state functional MRI data (300 dynamics). Spin-echo diffusion sequences were used with an echo planar imaging readout (TR/TE = 3000/33ms, 4 shots, resolution = $0.333 \times 0.333 \text{ mm}^2$) with b value = 1000 s/mm^2 (30 directions) for DTI data acquisitions. Identical FOV ($32 \times 32 \text{ mm}^2$) five slices (in 3mm thickness) were selected for both studies. The Fisher transformed z-scores of correlation coefficients of resting state fMRI signal (0.01-0.1Hz) between dorsal-to-dorsal and ventral-to-dorsal gray matter horns were quantified using custom code (AFNI, [1]). The diffusion parameters (FA&RD) at each axial slice were evaluated using a non-linear tensor fitting model (CAMINO). Post lesion data were acquired at two post-injury time points: recovery stage 1 (1-2 weeks) and stage 2 (6-9weeks). Twenty-four resting state functional (2 runs/animal, fMRI) and twelve DTI datasets (1 run/animal) were analyzed.

Results

Placements of five axial slice for the data acquisitions are shown in Figure A with a high resolution MTC image obtained from one monkey 2 weeks after a unilateral dorsal column injury in the background. The center slice is positioned around the injury at the left dorsal column (red arrow). A schematic view of the region of interest (ROI) placements in the white (top) and gray matter are shown in Figure B. On each slice, two ROI pairs were selected for rsFC measurements (1. left-right dorsal (LD-RD) and 2. left dorsal-ventral (LD-LV) horns). We analyzed the variations of the FA and RD values of the left dorsal white matter tract over time. Figure C&D show the mean and standard error of z-scores of the correlation coefficients for ROI pairs of 1&2(LD-LR&LD-LV) along the spinal cord segments (slices 1-5) respectively. Figures E&F demonstrate the similar temporal variations of FA and RD on the injured dorsal tract. Statistically significant differences between pre-injury (blue line) recovery stage 1 (red line) and stage 2 (green line) were measured using ANOVA1 (** p < 0.005, * < 0.05, indicated by different color stars). Resting state z-

Figures



(A) Horizontal anatomic magnetization transfer contrast (MTC) image shows the slice placement for fMRI and DTI acquisition. (B) Schematic illustration of the targeted unilateral dorsal column lesion (red patch with scissor) and selections of region of interests for fMRI connectivity analysis (red circles) and white matter tracts: dorsal right (DR, dorsal left (DL), lateral left (LL) and lateral right (LR). (C, D) Z-score of correlation across the spinal segments (15mm in length, 5 slices) between left-right dorsal horns (C, LD-RD) and left dorsal-ventral horn (D, LD-LV) at three different time points. (E, F) FA (E) and RD (F) across spinal segments.

scores showed significant differences between pre-injury and recovery stage 1 in comparison to stage 2 in the slice 3 (injury level) and adjacent slices. Additionally, we observed a strong correlation between the mean rsFC z-score (left-right dorsal horn) and the mean RD and AD at the early stage of recovery. The temporal variation of DTI parameters in three other white matter tracts are under investigation

Discussion

This study measured the effects of SCI on axonal integrity of the injured white matter tract and demyelination as well as rsFC between dorsal-dorsal and dorsal-ventral horns and their alterations during the process of recovery. Temporal changes in functional and DTI parameters in combination across spinal segments along the cord are being correlated with behavioral deficits and may be considered as useful predictors and sensitive imaging biomarkers of behavioral recovery.

Acknowledgements

This work was supported by a National Institutes of Health grant R01 NS09296 DoD grant W81XWH-17-1-0304 (Gore). We would like to thank Mr. Fuxue Xin and Mrs. Chaoui Tang for their assistance in acquiring the MRI data and animal care.

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Validation of Structural, Molecular, and Functional Imaging Biomarkers

SC160154 Spinal Cord Injury Research Program

W81XWH-17-1-0304



PI: John Gore, Ph.D.

Org: Vanderbilt University Medical Center

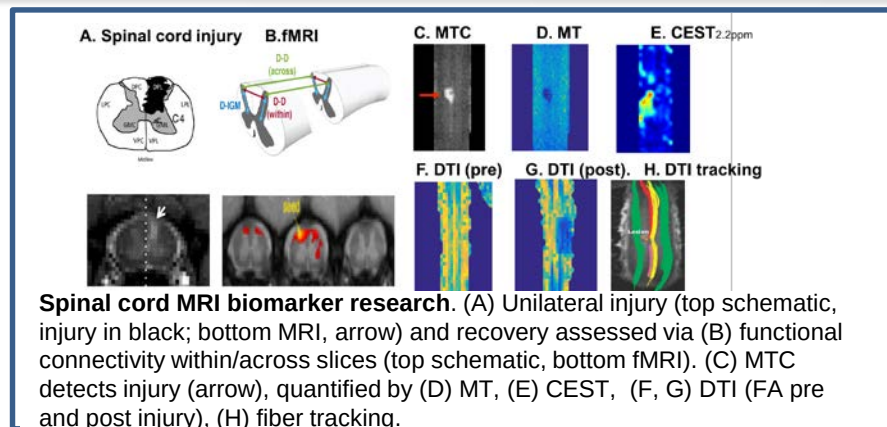
Award Amount: \$783,554

Study/Product Aim(s)

- Determine how the functional integrity of injured spinal cord grey matter repairs over time using functional MRI.
- Determine how injured spinal cord white matter repairs over time using structural and molecular MRI.
- Validate our MRI findings with invasive, gold-standard measures. These will include electrophysiology, behavioral measures and quantitative analyses of histological sections obtained at different time points during the course of recovery.

Approach

- We will implement sub-millimeter fMRI and multiparametric MRI (CEST, qMT, DTI) at 9.4 Tesla with optimized acquisition sequences and post-processing algorithms to measure functional connectivity, molecular changes, and structural alterations in normal NHP spinal cord and after injury and recovery in non-human primates (NHP). fMRI/MRI will be validated with electrophysiology, behavior, and histology.



Accomplishments: We have acquired and analyzed BOLD resting state fMRI, structural and molecular MRI (MT, CEST, DTI), and behavior data pre-injury and at regular intervals up to 6 months post-injury in NHPs. These in vivo biomarkers are being validated with electrophysiology and histology in NHPs.

Timeline and Cost

Activities	CY	17	18	19	20
fMRI, MRI in NHPs; first cohort of SCI with validation.					
Implement and validate in SCI.					
Implement and validate in SCI.					
Estimated Budget \$783,554		\$264,385	\$312,903	\$206,566	(projected)

Goals/Milestones

CY17 Goals – Refinement of spinal cord (SC) fMR/MRI methods; implementation and validation in first SC injury (SCI) cohort

- ☒ Implement fMRI/MRI on NHP, validate with electrophysiology.
- ☒ Implement fMRI/MRI in SCI; validate (recordings, histology).

CY18 Goals – Implementation and validation of SCI recovery

- ☒ Implement fMRI/MRI in SCI and follow recovery over a 6 month period; validate (recordings, histology).

CY19 Goals – Implementation and validation of SCI recovery

- ☐ Implement fMRI/MRI in SCI and follow recovery over a 6 month period; validate (recordings, histology).
- ☐ Identify biomarkers most sensitive to SC injury and recovery.

Comments/Challenges/Issues/Concerns

- Delay in ACURO and local IACUC approval until Sept. 16, 2018.

Budget Expenditure to Date

Projected Expenditure: \$540,001

Actual Expenditure: \$577,288

Updated: Nashville, TN, USA July15, 2019