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14. ABSTRACT The research performed aimed to 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. The major goal was to establish their relationships to gold-standard invasive measures of function and structure in non-human primates. The long term aims of the research are 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. We used a combination of advanced MRI methods to obtain 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. We determined how these changes predict and correlate with behavioral recovery and histological endpoints. Specific findings include: (i) measurements of functional connectivity by MRI reveal intrinsic neural networks within spinal cord that can be analyzed into several discrete sub-components; these components connect across segments, and within a segment there is a set of complex between-component connectivities; (ii) acute injury disrupts the rsFC of intraspinal circuits in grey matter, mainly below the injury, but these later return to their pre-injury strengths; these circuits thus have direct relevance to behavioral functions; (iii) the MRI measures of connectivity correspond closely to coherences between electrical local field potential (LFP) recordings from the same regions; (iv) multi parametric MRI including Chemical Exchange Saturation Transfer imaging can be used to map the acute release and later reduction of metabolites, while Diffusion Tensor Imaging and Quantitative Magnetization Transfer imaging can be used to track the extent of axonal disruption and demyelination and the spatiotemporal trajectories of recovery in individual white matter tracts, as confirmed by histology. These changes in MRI measures correlate strongly with the severity of initial behavioral deficits and subsequent recovery of hand use behavior and sensorimotor functions in the monkeys.					
15. SUBJECT TERMS spinal cord injury, MRI, fMRI, imaging biomarkers, non-human primates, functional connectivity					
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[1] INTRODUCTION: This research proposed to 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 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 currently 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 used a combination of novel, advanced MRI methods that provide unique insights into SCI progression, including functional integrity of grey matter and micro-structural and compositional changes in white matter to assess changes in structural, functional, and cellular/molecular properties of SCI over time in a monkey model. We also proposed to 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, spinal cord, spinal cord injury, cervical, imaging biomarkers, non-human primates, functional connectivity

[3] ACCOMPLISHMENTS:

Our SOW identified the following major goals:

Major Task 1

- (a) Implement high resolution fMRI on NHPs: implement sub-millimeter fMRI at 9.4Tesla along with appropriate acquisition sequences and post-processing algorithms for high quality measures of connectivity in normal animal. 100% Completed Y1
- (b) Map fMRI evoked responses to touch and heat: Map relevant sensory responses at high resolution to identify candidate seed regions in 3 pre-lesion (normal) animals. 100% Completed Y1
- (c) Acquire resting state fMRI data in 3 normal NHPs. Quantify fMRI connectivity by seed and data-driven methods: Perform connectivity analyses to identify circuits and degree of connectivity between horns, within and across segments. 100% Completed Y1
- (d) 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. 100% Completed Y2&3
- (e) Quantify changes in connectivity longitudinally in injured NHPs: Measure connectivity strengths and patterns from fMRI within and across segments at each time point imaged. 100% Completed Y2&3
- (f) Analyze and publish data. 75% complete Y3

Milestones for Y1: Completion of first animal cohort (at 9 months) on July 2, 2018
Milestones for Y2: Completion of two animal cohorts (at 15 and 21 months) on May 30, 2019
Milestones for Y3: Completion of final cohorts September 14, 2020
Milestones for Y3: Completion of analysis for final manuscripts (in progress)

Major Task 2

- (a) Implement high resolution MRI on NHPs: implement sub-millimeter CEST, qMT, and DTI acquisition sequences at 9.4T. 100% Completed Y1
- (b) Implement appropriate post-processing algorithms to derive parametric maps from CEST, qMT and DTI acquisitions. 100% Completed Y1
- (c) 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. 100% Completed Y2&3
- (d) Analyze and publish data. 80% complete Y3

Milestones for Y1: Completion of first animal cohort (at 9 months) on July 2, 2018
Milestones for Y2: Completion of two animal cohorts (at 15 and 21 months) on May 30, 2019.
Completion of manuscript (Wang et al. 2018; Wang et al. 2019)
Milestones for Y3: Completion of manuscript, published (Mishra et al.2020)
Milestones for Y3: Completion of manuscript, published (Lu et al. 2020)
Milestones for Y3: Completion of analysis for manuscript (Wang et al. 2020 submitted)

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. Analysis of immunohistological results from 6 cases remain to be completed. 90% complete Y3
- (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. Recordings have been completed, but analysis is ongoing from 4 cases. 70% complete Y3
- (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. 100% Completed Y3
- (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. Reactions for cytochrome oxidase and myelin have been completed. Immunohistological processing of sections for astrocytes and microglia has not been completed for 4 cases. We expect results to be similar to previous cases, but we will take advantage of the opportunity to examine individual subject variability. 90% complete Y3.
- (e) Analyze and publish data. 75% complete Y3

Milestones for Y1: Completion of first animal cohort (at 9 months) on July 2, 2018. Completion of manuscript (Wu et al. 2019)

Milestones for Y2: Completion of two animal cohorts (at 15 and 21 months) on July 18, 2019

Milestones for Y3: Completion of analysis for manuscripts, in progress

What was accomplished under these goals?

Several of our major accomplishments are described in our recent publications (Wang et al., 2018, 2019, 2020; Mishra et al., 2020; Wu et al., 2019) and others in preparation. A major goal of our current grant was to detect and measure inter-regional correlations in resting state fluctuations of magnetic resonance (MRI) signals within the spinal cord (SC), and to validate their neuronal and anatomical bases as measures of functional connectivity (rsFC). We have made significant progress in demonstrating the functional relevance of rsFC and in relating BOLD measurements to electrophysiology and behavior. We have also documented how targeted injuries to the spine evolve over time and may be interpreted as biomarkers of recovery.

Using functional MRI, we have found the resting state networks within and between slices show a more complex structure than heretofore realized. Our imaging studies have revealed more complex within- and between- segment correlations than previously reported. We have confirmed that these networks are directly affected by damage to the cord and, in our animal model, that modifications to the circuits correspond to behavioral changes in, for example, skilled hand use. In parallel we have documented changes in other MRI parameters and are currently performing analyses to integrate these different information into an overall predictive model of recovery. The major findings from current studies include:

- (i) Using data-driven Independent Components Analysis (ICA), rsFC by MRI reveals intrinsic neural networks within spinal cord that can be analyzed into several discrete sub-components; these components connect across segments, and within a segment there is a set of complex between-component connectivities.
- (ii) 7 discrete regions of locally high connectivity within each segment show significant resting state connectivity with other parts and between segments, and may be considered nodes of intraspinal networks.
- (iii) Acute injury disrupts the rsFC of intraspinal circuits in grey matter, mainly below the injury, but these later return to their pre-injury strengths; these circuits thus have direct relevance to behavioral functions.
- (iv) fMRI rsFC corresponds closely to coherences between electrical local field potential (LFP) recordings from the same regions; BOLD fMRI therefore reflects underlying neural activity.
- (v) Chemical Exchange Saturation Transfer (CEST) imaging can be used to map the acute release and later reduction of metabolites which we believe are dominated by excitotoxic glutamate.
- (vi) Diffusion Tensor Imaging (DTI) and Quantitative Magnetization Transfer (qMT) can be used to track the extent of axonal disruption and demyelination and the spatiotemporal trajectories of recovery in individual white matter tracts, as confirmed by histology.
- (vii) Changes in MRI measures correlate strongly with the severity of initial behavioral deficits and subsequent recovery of hand use behavior and sensorimotor functions in the monkeys.

ICA analysis of rsFC from MRI

We extended our original seed-based measures of intra- and inter-slice BOLD correlations using independent components analysis (ICA), a data driven approach used widely in brain fMRI. All functional images obtained from different monkeys were co-registered to a customized template in order to perform group level analyses. After standard pre-processing (motion and physiological noise corrections, bandpass filtering) group spatial ICA was performed by temporal concatenation of all the data using GIFT software and 34 components (across 5 slices) were visually identified as representing regions of highly synchronized and localized activity. Fig. 1 shows the spatial distribution of the 7 regions identified from ICA analysis of resting state signals acquired across 5 typical monkeys. These were used as seed regions of interest (ROI) to quantify between-ROI connectivities. Fig. 2 shows significant intra-slice between ROI connectivities averaged over 5 slices. Note the mean r values of all left sided intra-slice ROI pairs (i.e., LR-LV or LV-IL) are higher than their corresponding right pairs. All five monkeys were left hand dominant.

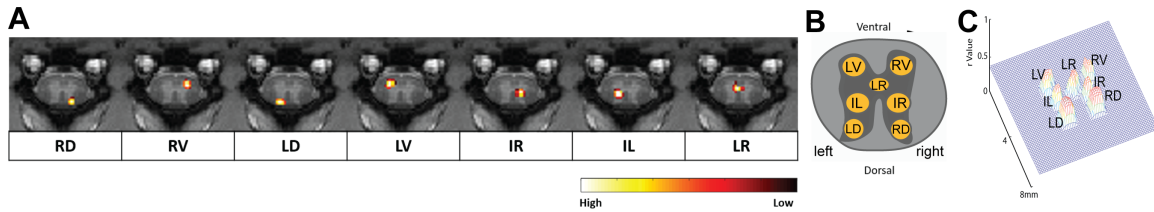


Fig. 1 A: Spatial distribution of 7 regions from representative slice 5 showing synchronized component ROIs obtained by ICA (Right Dorsal Horn (RD), Right Ventral Horn (RV), Left Dorsal Horn (LD), Left Ventral Horn (LV), Intermediate Right (IR), Intermediate Left (IL) and Left-Right (LR) junction between two horns.). B: Schematic showing the location of the seven regions identified. C: 3D plots.

We applied graph theory analysis to characterize network properties of the ICA components. Fig. 3 shows the connectivity matrix of 7 nodes and 12 edges within one segment.

Inter-slice correlations between regions were also robustly detectable. As a function of distance between segments, nearest slices are most strongly correlated for ROI pairs, and for the 4 horns, the interslice connectivity reduces by $\approx 50\%$ after three slices.

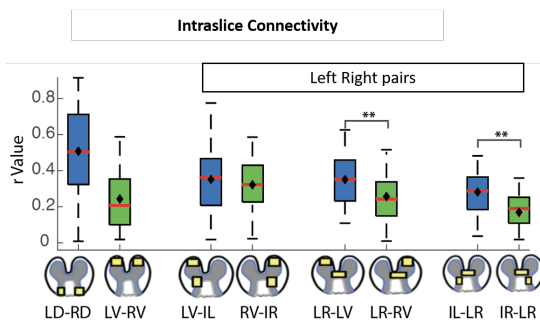


Fig. 2 Group box plots of intra-slice connectivity of significant ROI pairs (LD-RD, LV-IL, RV-IR, LR-LV, LR-RV, IL-LR) averaged over 5 slices for 5 monkeys. Connectivity was stronger in left versus right ROI pairs (*: $p < 0.05$). Schematics below the box plots show the ROI pairs compared.

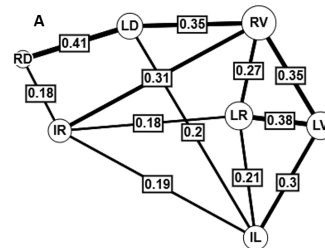


Fig.3 Graph theoretical model of connectivities within a single segment showing 7 nodes with 12 edges. Numbers in boxes denote connection strengths, the thickness of edges is proportional to the weights between nodes and the node size is proportional to its strength.

Across segments, a graph theory analysis revealed four communities or hubs, which are subdivisions of non-overlapping groups of nodes within the network, as shown in Fig. 4. A community is formed from similar nodes of different slices such as the left and right dorsal horn of slice1 and slice 2 (white nodes in Fig. 4). The behavior of nodes within a community can be understood when shown on a connectivity matrix arranged as in Fig 4. It can be seen that the number of edges/connections is greater between nodes of the same community than between communities, but each community spans more than one segment.

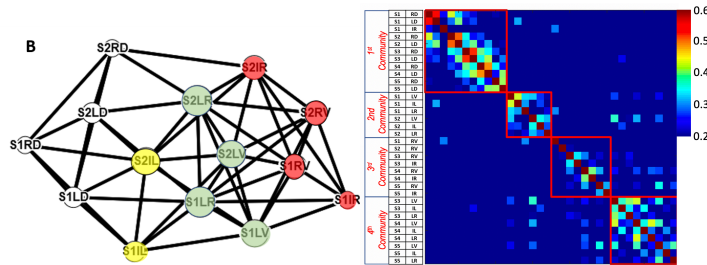


Fig. 4 Graph theory analysis of ICA data. Left: a network representation of two representative slices denoted by S1 and S2. 4 communities of nodes (in different color groups) are formed. Right: The connectivity matrix of all the components from all slices arranged such that the nodes from same community are next to each other. Each red box

Validation of stimulus-driven fMRI signals with electrophysiology.

To validate the interpretation of fMRI signals and rsFC, we recorded stimulus-evoked and spontaneous spiking activity and local field potentials (LFPs) from each monkey's spinal cord. We first validated the fMRI activation with well-documented dorsal horn responses to tactile stimulation of a distal finger pad and found that both BOLD and electrophysiological signals elicited by tactile stimulation co-localized to the deeper lamina of 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 primary somatosensory cortex (area 3b). Fig. 5 shows that 8-Hz innocuous tactile stimulation of a single digit elicited strongest BOLD signal changes in the ipsilateral dorsal horn, with a peak value of 0.76 %. Fig. 6 shows representative stimulus-driven LFP and multi-unit 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. 6B and frequency peaks are apparent at harmonics of 8Hz in the LFP power spectra (Fig. 6C). Group normalized LFP power changes showed digit-selective responses to the tactile stimulus (compare the four different color curves in Figs. 6A, 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 (Figs. 6H, K). Group normalized spike rates obtained from four different recording sites (left D3, right D3, left D5, and right D5) are presented in Figs. 6G, J. Overall, significant and correlated changes in BOLD, LFP and MUA were observed in the ipsilateral dorsal horn of the somatotopically appropriate cervical segment.

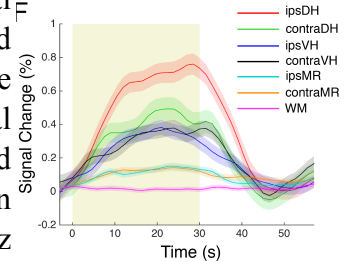


Fig. 5 Averaged BOLD signal produced by tactile stimulation (shaded yellow) of single digit. Dorsal horn (DH) Ventral horn (VH). Middle/intermediate gray matter(MR) that are ipsilateral (ipsi) and contralateral (contra) to the stimulus. White matter (WM) control region.

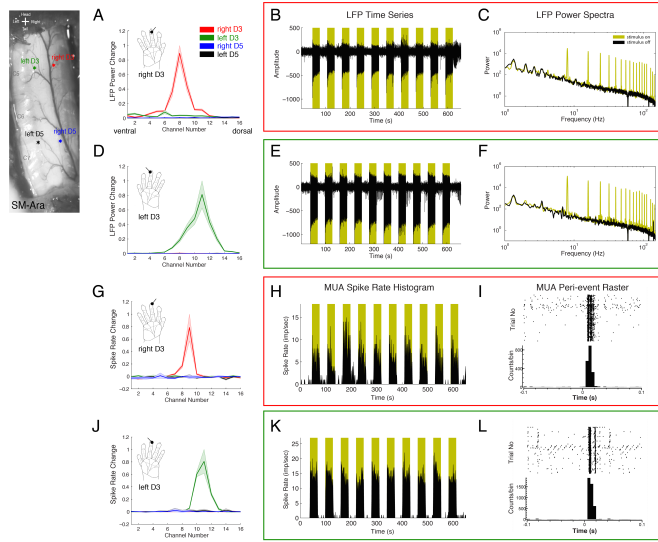


Fig. 6 Stimulus-driven LFP and spike activity responses in one representative monkey. A,D: Averaged LFP power changes computed from Welch's power spectra, and (G, J) spike rate changes between 30s stimulus-on and -off in four electrodes under right-D3 and left-D3 innocuous 8Hz tactile stimulus conditions. Shaded error bars = 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 largest changes and their corresponding (I,L) raster plots (top row) and peri-event histograms (bottom row).

Local dorsal spatial rsfMRI profiles.

We plotted the local correlation profiles of the rsfMRI signals from a dorsal horn seed region along the dorsal-intermediate-ventral axis. Small regions of interest (ROIs) were drawn from the dorsal towards the ventral horn on one side of the spinal cord (Fig. 7A). We found 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. 7B). The temporal signal noise ratio (tSNR) of the voxels examined did not show changes as a function of distance from the seed suggesting the correlation profile was not driven by varying noise levels across three zones.

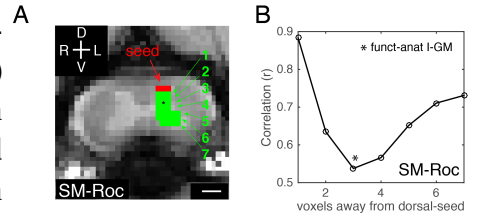


Fig. 7 Dorsal to ventral correlation profile. A: ROIs defined on one side of the spinal cord. Mean time series of the dorsal region (red voxels) was used as seed to compute mean rsfMRI with voxels from each depth (green voxels). Scale bar = 1mm. B: Observed U-shaped profile. * represents defined intermediate GM (I-GM) seeds

Validation of rsfMRI connectivity of the spinal horns.

Inter-horn resting-state connectivity patterns observed in rsfMRI were compared to LFP connectivity patterns derived by analyzing broadband coherences between signals from different elements in each linear array electrode. Connectivity measures between different specific pairs of ROIs were compared: dorsal-dorsal (within-slice), dorsal-intermediate gray matter and dorsal-dorsal (across-slice) (Fig. 8A). At the group level (N=12 for both 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.s 8B-C). Moreover, LFP coherence was found to be statistically significant at depths (from dorsal surface) up to 1.5 mm between contralateral regions at precisely the same depth. For monkeys that underwent electrophysiology and fMRI recordings, linear regression between electrophysiology coherence and rsfMRI correlation gave a r -value of 0.5079 ($p=0.0058$). The

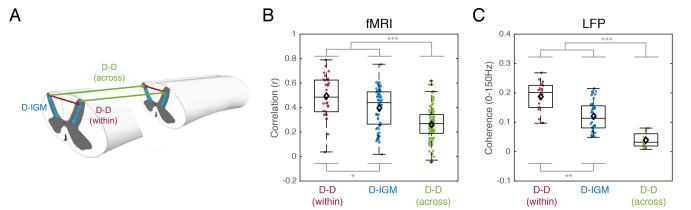


Fig. 8 Comparison between resting-state fMRI and LFP connectivities. A: Schematic diagram of correlation and coherences computed in the recordings of the spinal cord. B,C: Group averaged boxplots of connectivity measures displayed as Pearson's correlation for fMRI and averaged coherences for LFP.

within-slice resting-state connectivity was robust between horns, but across-slice correlation strengths were found to be consistently lower in these animals (mean BOLD $r=0.26$ and LFP coherence=0.04). Trends of connectivity measures for different ROI pairs (Fig. 8B-C) were also highly similar between the two modalities. From our overall electrophysiology studies we conclude that the pattern of LFP coherences matched that of the rsFC from MRI, and BOLD signal magnitudes directly reflected electrical activities.

Effects of injury on resting state fMRI signals

We hypothesized that rsFC would be changed after an injury that is known to alter spinal cord function and behavior. Animals were subjected to a targeted unilateral dorsal cord lesion that deprives the sensory inputs to the brain and they were imaged and their behaviors were assessed at different times after the injury. For reporting purposes, the post-lesion period was divided into three phases: the beginning (recovery stage, 1), middle (recovery stage 2) and end (recovery stage 3) of the recovery period (2-24 weeks). Fig. 9 shows that the difference between pre-lesion and post-lesion stages was most apparent in the lower frequency components of the IC power. After injury, the spectral power of rsfMRI signals in the low frequency range (0.01-0.033 Hz) dropped drastically (Fig 9A) and never returned to pre-lesion level. (Fig 9B). Importantly, injury-induced changes in intra-slice connectivity varied for below and above segments. For below injury slices, connectivities were significantly weakened for LD-RD, LV-RV and LR-LV (among the significant connectivities obtained). For the above injury slices, there were drops in LD-RD connectivity strength, but significant increase for LV-RV and LV-LR in connectivity (signifying recovery) post injury.

Multi-parametric MRI provides complementary information of structure and composition

We also acquired, in the same sessions, multi-parametric images that report different aspects of the effects of interventions and help interpret the results.

(i) Diffusion parameters vary following injury and correlate with functional metrics and behavior. We acquired diffusion tensor images at several time points before and after injury in each animal in order to identify which specific white matter tracts were injured, and how those structural injuries affected rsFC in the cord. Full details are described in our recent paper (Mishra et al., 2020). We quantified the radial diffusivity (RD), axial diffusivity (AD), mean diffusivity (MD) and fractional anisotropy (FA) regionally and at each time point. RD, AD and FA values in neighboring segments of the lesioned dorsal tract indicated prominent disruptions

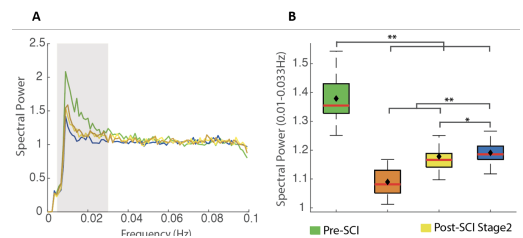


Fig. 9 A: RsFC measured as integrated power vs frequency. B: box plots at different stages after injury of power in the frequency range 0.01-0.033 Hz.

in fiber orientation, integrity and myelination. FA, RD and AD values of the lesioned dorsal and non-lesioned lateral tract on the lesion side, as well as those in the lesion site, closely reflected the behavioral recovery. Fig. 10 shows the results of comparing metrics of behavioral recovery on a hand usage task with tract specific diffusion parameters. Fig. 10 shows that the DTI parameters and the functional connectivity between specific horns showed strongly similar time courses post injury. These results provide further confirmation that measurements of rsFC and DTI reflect behaviorally-relevant structural and functional changes in integrity of the spinal cord.

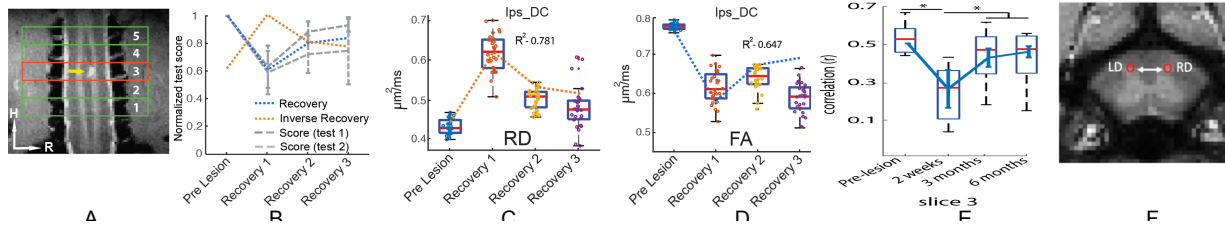


Fig. 10 Changes in DTI parameters, rsFC and behavior before and after SCI. A: Coronal slice showing axial slice placement for DTI and fMRI data acquisitions. B: Normalized test scores of behavioral performance in food retrieval test pre and post recovery Score 1= success rate, Score 2 = number of flexes needed. Blue and orange dotted lines refer to predicted recovery in a regression model. C, D: Boxplots of changes in diffusion parameters and corresponding R^2 values for radial diffusivity (RD) and fractional anisotropy (FA) of lesioned dorsal tracts caudal to injury, with the behavioral recovery curves (dotted lines in the background). E: Boxplot showing the correlation (r) of resting state BOLD signal between LD-RD horns in one slice. F: Axial view showing left and right dorsal (LD&RD) horns.

(ii) QMT and CEST report changes in myelination and tissue metabolites A time-efficient quantitative magnetization transfer (qMT) acquisition was developed that provides parameters including pool size ratio (PSR), which assesses changes in the myelination of the spinal cord. PSR delineated normal white matter (WM), gray matter (GM) and abnormal tissues in the injured cervical cord. A significant decrease in PSR was observed both rostral and caudal to the lesion site ($p < 0.05$).

Chemical exchange saturation transfer (CEST) and nuclear Overhauser enhancement (NOE) effects were derived from Z-spectra to quantify changes in metabolites, mobile proteins and membrane lipids associated with the secondary injury and inflammation after SCI, and during the repair and recovery process. We found acute increases in a resonance at 3.5 ppm believed to represent glutamate that returned slowly towards normal over time. Our qMT and CEST results are reported fully in our publications (Wang et al., 2018; 2019; 2020).

Validation of the extent of injury with histology

Prior to final data collection sessions, a subset of animals received tracer injections into digit representations on each side of the spinal cord with two different tracers (fluororuby, biotinylated dextran amine [BDA]), as well as subcutaneous injections into the fingers on each hand with a third tracer (cholera toxin subunit B [CTB]). The spinal cord was also sectioned for further processing to reveal markers of tissue condition, such as myelinated fiber density and immunoreactivity to glial markers, particularly IBA1 for microglia, and GFAP for astrocytes. Pandemic restrictions has delayed the completion of additional monkey cases, as well as completing immunohistochemistry for a series of sections from 6 cases. The sections have been preserved frozen for processing when in-lab work resumes. The histological assessments show

demyelination was apparent in the rostral segments of lesioned dorsal columns in all animals studied to date. Fig. 11 shows one example. We found variability across animals in the degree of axonal degeneration examined 6 months after injury, so we have an opportunity to quantify how imaging biomarkers vary with histological markers and behavioral deficits.

Summary

Some data collected are still being analyzed, and further analysis will provide additional information on the interpretation of images from histology and behavior. Our publications to date reveal a comprehensive description of the manner in which various MRI metrics evolve as a result of SCI. We have established a further aim to develop an algorithm by which data derived from quantitative, non-invasive, multimodal MRI can be integrated and used to reliably monitor and predict the recovery of injured spinal cord, and to thereby evaluate the effects of therapies and interventions. We have looked at the changes that occur in individual MRI parameters and now further hypothesize that appropriate combinations of MRI techniques (fMRI, DTI, CEST, qMT) are capable of tracking the spontaneous repair of injured spinal cord by assessing changes in the micro-structural integrity and anatomical fiber connectivity in white matter tracts, their degree of myelination, intrinsic changes in the composition of damaged tissues, as well as the functional integrity of spinal grey matter, by assessing the patterns of intra-spinal functional connectivity. Our work has established the strong correspondence between non-invasive fMRI activation and connectivity metrics with invasive electrophysiology and post-mortem histology of injected tracers in a well controlled, realistic monkey model of SCI. RsFC is thus a reliable surrogate for neural activity and intra-spinal cord networks, while DTI and qMT report changes in white matter structure and composition and CEST reflects levels of small metabolites. We believe that an appropriate combination of these features will provide accurate descriptions of the functional and structural integrity of the cord at different stages and can be used in a statistical multivariate model as an objective, sensitive indicator for assessing and predicting recovery

ANIMAL CARE PROTOCOLS:

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Submitted to IACUC: 03/20/2019 Approved by: 05/02/2019 [IACUC protocol M1600079-00]

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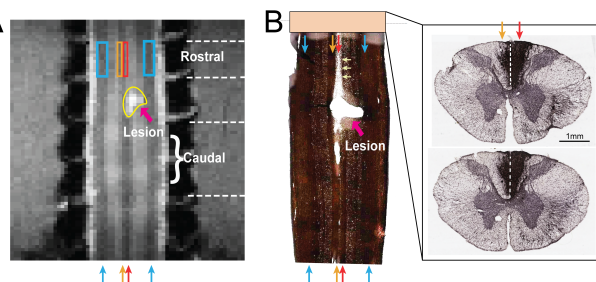


Fig. 11 Histological evaluation of spinal cord injuries in two monkeys. A: MTC structural image shows the lesion and stripes of grey and white matter on coronal slices. B: Myelin fiber staining shows the lesion and the degenerated dorsal column tract. Axial images on the right show the high expression of microglia marker IBA1 on the dorsal column tracts.

Submitted to IACUC: 04/03/2018 Approved by: 05/02/2018 [IACUC protocol M1600079-00]

ACURO Approval: 09/26/2017

Submitted to IACUC: 03/09/2017 Approved : 08/15/2017 [IACUC protocol M1600079-00]

STATUS: ACTIVE

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

The project provided training opportunities for Biomedical Engineering graduate students in fMRI and electrophysiological methodologies and analyses, and training for an entry-level research faculty member. Professional development included submissions and presentations by research faculty team members at a major professional conference (International Society for Magnetic Resonance in Medicine in 2018, 2019, 2020).

How were the results disseminated to communities of interest?

Nothing to report at this stage beyond publications and conference presentations.

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

Follow-on-funding for this project has been requested from DoD and NIH.

[1] National Institute for Neurological Diseases and Stroke

direct costs over 5 years

Resting State Connectivity in Primate Spinal Cord

[2] Department of Defense Spinal Cord Injury program

over 3 years

Structural, molecular and functional imaging biomarkers in spinal cord injury in non-human primates

What will be the next step(s) for this project?

We are proposing new Aims to further this research:

Specific Aim 1. Demonstrate multi-parametric MRI provides behaviorally-relevant biomarkers of injury and predicts functional outcomes in a clinically-relevant contusion model of spinal cord injury. In particular we will develop a multivariate model to predict tissue recovery by integrating imaging parameters and validate the results with functional behavioral measures.

Specific Aim 2. Demonstrate multi-parametric MRI biomarkers are reliable indicators of the effects of treatments in a clinically-relevant contusion model of spinal cord injury.

We will demonstrate the sensitivity and specificity of MRI biomarkers for evaluating the effects of two therapies: (i) the neuroprotective drug Riluzole and (ii) the neuroregenerative drug Cethrin. Riluzole suppresses the release of glutamate and cytokines associated with inflammation in the acute phase after injury, whereas Cethrin promotes regeneration and remyelination of

damaged axons in the subacute phase. We will correlate changes in MRI parametric images with improvements in behavior and function comparing treated with untreated animals.

Specific Aim 3. Validate the interpretation of MRI biomarkers using localized measurements of gene expression, histology and proteomic changes in injured tissues, and the functional relevance using behavioral metrics. We will extend our previous use of histology to perform spatiotemporal microarray analysis of spinal tissues at and around the injury epicenter to measure gene expression profiles, and directly compare these to the corresponding MRI measures in individual animals. We will also perform imaging mass spectroscopic imaging of tissue sections, co-registered with in vivo MRI data, to identify patterns of protein expression within the tissues. We expect to be able to link differences in gene and protein expressions between treated and untreated conditions to differences in corresponding MRI CEST, DTI, qMT, and fMRI measures, and ultimately to the behavioral outcomes of individual subjects.

How would you classify your lead candidate product?

(b)Diagnostic - our product is an imaging protocol that can assist the accurate diagnosis of the extent and severity of an injury

(d)Research Tool to Address a Research Bottleneck - our protocol can assist the development of new therapies because at present there are no ways to monitor the effects of treatment objectively using non-invasive imaging.

(e)Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.) - our protocol and imaging methods can be translated to clinical practice to improve the management of patients

How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

The military relevance of research into spinal cord injuries and methods for assessing their recovery and treatment is underscored by the existence and mission of the CDMRP SCIRP program. The Vision of this program is to “advance the treatment and management of spinal cord injury and ameliorate its consequences relevant to injured Service members”. From their website, the SCIRP focuses its funding on innovative projects that have the potential to make a significant impact on improving the function, wellness, and overall quality of life for military service members as well as their caregivers, families, and the American public. This research directly addresses those aims.

SCI is one of the most prevalent causes for permanent disability of military personnel (and their family members) in the United States, and is often the result of a sudden, traumatic, and life-changing event. Traumatic SCI can lead to sensorimotor and autonomic nerve damage, potentially resulting in long-term disability. SCI has devastating consequences for patients due to severe impairments of motor and/or sensory functions, and has a significant impact on quality of life, life expectancy and economic burden. Better methods and noninvasive objective biomarkers are needed to guide the clinical treatment of SCI and the evaluation of novel therapies. Improved understanding of the spontaneous repair and adaptive plasticity changes after injury provide

critical information for developing mechanism-based therapy and for guiding selection of SCI patient in clinical trials.

A recent paper by Schoenfeld et al. (*Spine*, 2013, 38(20): 1770-8) highlights the increasing prevalence of spinal cord injuries directly as a result of combat. In a retrospective study of military injuries over the period 2005 to 2009, they noted “872 (11.1%) casualties with spine injuries were identified among a total of 7877 combat wounded. The mean age of spine casualties was 26.6 years. Spine fractures were the most common injury morphology, comprising 83% of all spinal wounds. The incidence of combat-related spinal trauma was 4.4 per 10,000, whereas that of spine fractures was 4.0 per 10,000. Spinal cord injuries occurred at a rate of 4.0 per 100,000. Spinal cord injuries were most likely to occur in Afghanistan (incident rate ratio: 1.96; 95% confidence interval: 1.68-2.28), among Army personnel (incident rate ratio: 16.85; 95% confidence interval: 8.39-33.84), and in the year 2007 (incident rate ratio: 1.90; 95% confidence interval: 1.55-2.32). Spinal injuries from gunshot were significantly more likely to occur in Iraq (17%) than in Afghanistan (10%, $P = 0.02$).” They conclude “the incidence of spine trauma in modern warfare exceeds reported rates from earlier conflicts.”

Combat injuries are not the only cause of spinal cord injuries - they can arise from other types of trauma including vehicular accidents and falls. They occur often in younger, physically active individuals and thus there are long term consequences for the affected subjects and their families.

From the research proposed, there will be direct clinical benefits of having a method to track changes in cords after injury and during treatment, and to provide new information on cord function and structure. Such information is critical for evaluating the efficacy of individual treatment programs, and for determining the optimal time window, targets and effectiveness of therapeutic interventions. Direct validation of MRI biomarkers on evaluating response to a promising pharmacologic treatment of acute SCI would facilitate new drug discoveries and have direct impact on acute SCI patients. All patients with traumatic SCI are likely to benefit from the understanding obtained of the nature of spontaneous repair, and individual patients will benefit by using MRI during the course of their treatment. The imaging procedures and identified pathology-specific biomarkers can be readily adapted to clinical scanners available throughout the health care system and provide objective non-behavior surrogates in clinical trials.

Transition Plan

The imaging methods developed and validated under this program of research can be immediately translated, with appropriate modifications, to human MRI scanners for applications to human subjects in clinical environments. The high fields we use to develop and validate methods in non-human primates afford some technical advantages when studying smaller primates but in principle equivalent information can be obtained in human subjects at the clinically available field strength of 3 Tesla using specialized spine RF coils and lower spatial resolution, a change that does not sacrifice essential information given the larger sizes of human cords compared to monkeys. Note that there are available also higher field (7 Tesla) human scanners, including one at our institution, which are entering into clinical practice as FDA-approved commercial products, and where these are available they may be preferred and used. However, our focus for translation will be to develop and translate the imaging methods to 3T

scanners that are in more routine use clinically. This will require modification and optimization of imaging protocols on commercial scanners. Our current institutional expertise includes the ability to program clinical scanners from Philips Healthcare, where we will start. However, we see no major difficulties in implementing these methods on other vendor platforms including GE and Siemens, which collectively account for the vast majority of the MRI market worldwide.

The feasibility of implementing these methods at 3T has already been established. For example, resting state functional connectivity of human spine networks, qMT, DTI and CEST have each been individually implemented on our 3T scanners and used to examine spinal cords in normal subjects or those with multiple sclerosis. For example, our institute has recently published separate illustrations of each imaging method e.g.

(i) DTI - “Diffusion MRI microstructural models in the cervical spinal cord - Application, normative values, and correlations with histological analysis”. Schilling KG, et al. *Neuroimage*. 2019 Nov 1;201:116026. doi: 10.1016/j.neuroimage.2019.116026.

(ii) qMT - “Selective inversion recovery quantitative magnetization transfer imaging: Toward a 3 T clinical application in multiple sclerosis.” Bagnato F, et al. *Mult Scler*. 2020 Apr;26(4):457-467. doi:10.1177/1352458519833018.

(iii) FMRI - “A practical protocol for measurements of spinal cord functional connectivity.” Barry RL, et al. *Sci Rep*. 2018 Nov 8;8(1):16512. doi: 10.1038/s41598-018-34841-6.

(iv) CEST - “Amide proton transfer CEST of the cervical spinal cord in multiple sclerosis patients at 3T.” By S, et al. *Magn Reson Med*. 2018 Feb;79(2):806-814. doi: 10.1002/mrm.26736.

These reports demonstrate that we are technically capable to implement these methods on a clinical 3T scanner. Further work (taking approximately 6 months) would be needed to fully optimize the imaging acquisitions and translate analytical methods to a reliable, convenient package for routine use on clinical systems. Also, many SCI patients have metallic implants (such as screws) within the regions to be imaged and these can affect the image quality and produce artifacts. However, using state-of-the-art RF pulse shapes and signal processing, imaging close to metal is now possible and such methods will be incorporated into our imaging protocols. We have extensive experience in the design of novel RF pulses with specific attributes, while commercial products are also available for near-metal imaging. Our specific plans would be:

(1) to translate robust, reliable methods to detect and quantify functional connectivity in human spinal using resting state fMRI images on 3T MRI scanners. Our experiences to date show this will be feasible with reduced physiological noise and the use of newly-available acquisition sequences such as inner volume imaging. The pulse sequence parameters need to be carefully chosen to satisfy the competing criteria of spatial coverage, high in-plane resolution, and sufficient temporal resolution, and will be iteratively improved upon as there is scope to optimize the acquisition sequence in terms of SENSE and multi band factors, echo train length, and voxel dimensions.

(2) to further develop and optimize post-processing strategies to derive resting state connectivity. We have previously used seed-based analyses but ICA and related methods provide data-driven results that are more objective. We also would wish to incorporate a spine template for co-registrations between images and sessions. Such tools are currently being developed e.g. the

spinalcordtoolbox available on GitHub <https://github.com/neuropoly/spinalcordtoolbox/tree/master/spinalcordtoolbox>.

(3) to implement optimized multi-parametric spine imaging protocol at 3T incorporating rsFC, DTI, CEST and qMT imaging in a cohort of subjects with spinal cord injuries. Using this protocol, human subjects can be enrolled into a clinical study in which functional metrics may be correlated with the imaging data. We would aim to validate the interpretation of functional connectivity and other MRI measurements within the spine as a biomarker of spinal integrity and for assisting clinical management decisions. Measurements of rsFC will be correlated with neurological assessments of function to demonstrate their clinical relevance. Conventional structural and novel multiparametric MRI will assist in detecting and quantifying the extent of cord damage. This may require a multi-center trial to accrue enough subjects for statistical power. The methods may also be incorporated into ongoing trials of novel therapies. These studies may be achieved using both standard of care imaging (in which case there are no additional significant costs) and via external grant funding from NIH or other source. Note that none of the “experimental” methods expose subjects to any additional significant risk and the acquisition parameters stay within the limits of exposure approved by the FDA for clinical scanners. As such the studies conform to all relevant regulations.

[4]. IMPACT:

The results are significant and will have practical impact because they demonstrate and validate how metrics derived from novel, quantitative, multi-parametric MRI acquisitions, can be used to assess specific pathophysiological changes in the spinal cord after injury, and thereby together be used as non-invasive biomarkers of repair and recovery. There is a widely recognized need for methods that can assess the status of injured cord tissues, monitor the effects over time of interventions designed to promote recovery, be used in the acute phase to predict outcomes of injury at later times, and have direct relevance to specific functions and behaviors. For translational SCI studies, reliable biomarkers of both spontaneous recovery/repair processes and the effects of new interventions or drugs are critical for designing more effective therapies and for evaluating treatment effects. Our results demonstrate how multi-parametric imaging data can be used to monitor and predict functional recovery. This information is of great importance and clinically relevant for studies of human SCI. Once successfully developed and validated, non-invasive multi-parametric MRI methods can be readily deployed for studying human spinal cord injury on clinical MRI scanners. Validation of MRI measures with invasive “gold-standard” measurements in non-human primates provides a firm basis for making inferences about changes in cord function and structure following traumatic injury, a knowledge that can be extrapolated to human MRI studies.

This is the first comprehensive, systematic evaluation of the sensitivity and specificity of multi-parametric MRI as reliable biomarkers of spinal cord injury and repair in a clinically-relevant injury model. In 3 years we have made significant progress in demonstrating the correspondence between quantitative MRI measurements and electrophysiology and function. Importantly, we have found the resting state networks within and between segments of the cord show a more complex structure than heretofore realized, but a structure amenable to innovative methods of analysis. We have confirmed that these networks are directly affected by damage to

the cord and that modifications to the circuits correspond to behavioral changes. We have found evidence that CEST imaging can be used to map the acute release and later reduction of excitotoxic glutamate, while DTI and qMT can be used to track the extent of axonal disruption and demyelination and the spatiotemporal trajectories of recovery in individual white matter tracts. These different metrics can be integrated into a multivariate model that can be used to assess the cord and predict outcomes. There will be direct clinical benefits of having a method to track changes in recovering cords and to provide new information on cord function and structure. Such information is critical for evaluating the efficacy of individual treatment programs, and for determining the optimal time window, targets and effectiveness of therapeutic interventions. All patients with traumatic SCI are likely to benefit from the understanding obtained of the nature of repair after injury, and individual patients will benefit by using MRI during the course of their treatments.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

There will be direct clinical benefits of having a method to track changes in recovering cords and to provide new information on cord function and structure. Such information is critical for evaluating the efficacy of individual treatment programs, and for determining the optimal time window, targets and effectiveness of therapeutic interventions. All patients with traumatic SCI are likely to benefit from the understanding obtained of the nature of spontaneous repair, and individual patients will benefit by using MRI during the course of their treatment.

These studies are directly translatable to human patients. If the monkey data confirm the interpretation of MRI as direct indicators of functionally and structurally relevant changes within the cord, the methods developed can be implemented on clinical 3T MRI scanners within a short period (≈ 1 year), and a clinical trial to validate the conclusions from non-human primates could be started. These methods could also be combined with other treatment trials to monitor the nature of treatments.

[5] CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

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

The global pandemic caused unavoidable delays in data collection and occasional delays in remote access to collected data to perform analyses. Safety protocols for in-person work were established when city and medical center guidelines allowed, and we were able to resume data collection to nearly complete the longitudinal studies. Difficulties in receiving some deliveries and supplies delayed some tissue processing proposed; however, the series of spinal cord and brain sections that have not been processed have been stored in cryoprotectant and frozen.

Changes that had a significant impact on expenditures

Nothing to report

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

Nothing to report

Significant changes in use or care of human subjects

Not Applicable.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

[6] PRODUCTS:

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

Journal publications.

Published and Funding Acknowledged

Mishra A, Wang F, Chen LM, Gore JC. Longitudinal changes in DTI parameters of specific spinal white matter tracts correlate with behavior following spinal cord injury in monkeys. *Sci Rep.* 2020 Oct 14;10(1):17316. doi: 10.1038/s41598-020-74234-2. PMID: 33057016; PMCID: PMC7560889.

Lu M, Wang F, Chen LM, Gore JC, Yan X. Optimization of a transmit/receive surface coil for squirrel monkey spinal cord imaging. *Magn Reson Imaging.* 2020 May; 68:197-202. doi: 10.1016/j.mri.2020.02.011. Epub 2020 Feb 19. PMID: 32087231; PMCID: PMC7136156.

Wang F, Wu TL, Li K, Chen LM, Gore JC. Spatiotemporal trajectories of quantitative magnetization transfer measurements in injured spinal cord using simplified acquisitions. *Neuroimage Clin.* 2019;23:101921. doi: 10.1016/j.nicl.2019.101921. Epub 2019 Jul 2. PMID: 31491830; PMCID: PMC6639592.

Wang F, Zu Z, Wu R, Wu TL, Gore JC, Chen LM. MRI evaluation of regional and longitudinal changes in Z-spectra of injured spinal cord of monkeys. *Magn Reson Med.* 2018 Feb;79(2):1070-1082. doi: 10.1002/mrm.26756. Epub 2017 May 25. PMID: 28547862; PMCID: PMC5702279.

Wu TL, Yang PF, 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. *Nat Commun.* 2019 Mar 29;10(1):1416. doi: 10.1038/s41467-019-09485-3. PMID: 30926817; PMCID: PMC6440970.

Submitted and Under Review

Wang F, Zu Z, Wu TL, Yan X, Lu M, Yang PF, Byun NE, Gore JC and Chen LM. CEST and NOE MRI of Injured Spinal Cord in Monkeys (revision submitted to *Neuroimage Clin*)

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

All presentations international except (1)

1. Tung-Lin Wu, Feng Wang, Arabinda Mishra, George H. Wilson III, Nellie Byun, Li Min Chen, and John C. Gore—Longitudinal Assessment of Spinal Cord Injury Using DTI and qMT. 2018 Biomedical Engineering Society Annual meeting. Atlanta, GA.*
2. Feng Wang, Tung-Lin Wu, Ke Li, Li Min Chen, John C. Gore. Rapid and robust high-resolution mapping of pool size ratio of spinal cord after unilateral dorsal column lesion in squirrel monkeys at 9.4T. 2018 ISMRM 26th Annual Meeting & Exhibition*
3. Arabinda Mishra, Feng Wang, Li Min Chen, John C Gore Quantitative Assessment of Temporal Changes in fMRI and DTI in the Squirrel Monkey Spinal Cord after Injury. 2019 ISMRM 27th Annual Meeting & Exhibition ISMRM*
4. Xinqiang Yan, Feng Wang, Ken Wilkens, Daniel Colvin, Li Min Chen, John C. Gore. Combined volume T/R and surface Rx-only coils for simultaneous brain and spinal cord imaging of squirrel monkey at 9.4 T 2019 ISMRM 27th Annual Meeting & Exhibition*
5. Feng Wang, Zhong-Liang Zu, Tung-Lin Wu, Xinqiang Yan, John C. Gore, Li Min Chen. Rapid and Accurate CEST and NOE MRI of Injured Spinal Cord Using Multi-pool Fitting. 2019 ISMRM 27th Annual Meeting & Exhibition*
6. Feng Wang, Junzhong Xu, Tung-Lin Wu, Pai-Feng Yang, Nellie E. Byun, Li Min Chen, John C. Gore. Structural Assessment of Injured Spinal Cord Using the Spherical Mean Technique and Diffusion Tensor Imaging. 2019 ISMRM 27th Annual Meeting & Exhibition
7. Li Min Chen, Feng Wang, Pai-Feng Yang, Tung-Lin Wu, Arabinda Mishra, Qing Liu, Nellie Byun, John C Gore. Progression and Recovery of Traumatic Spinal Cord Injury Assessed by Multiparametric MRI in Non-human Primates. Meeting: SCI 2020: Launching a Decade for Disruption in Spinal Cord Injury Research. 2019 NIH Bethesda, MD*
8. Pai-Feng Yang, Tang-Lin. Wu, N. Byun, John C. Gore, Li Min Chen. Altered neural activity and functional connectivity in injured cervical spinal cord in monkeys. 2019 49th Society for Neuroscience annual meeting *
9. Feng Wang, Tung-Lin Wu, Pai-Feng Yang, Nellie E. Byun, Li Min Chen, John C. Gore. Temporal features of behavioral impairment and recovery after spinal cord injury relate to Regional changes of magnetization transfer and diffusion MRI parameters over months period 2020 ISMRM 28th Annual Meeting & Exhibition*
10. Anirban Sengupta, Arabinda Mishra, Feng Wang, Li Min Chen, John C. Gore Intrinsic functional connectivity of spinal cord can be used to differentiate injured monkeys from normal using machine learning. 2020 ISMRM 28th Annual Meeting & Exhibition

*material in these presentations included in published manuscripts

- **Website(s) or other Internet site(s)**
Nothing to report
- **Technologies or techniques**

Lu M, Wang F, Chen LM, Gore JC, Yan X. Optimization of a transmit/receive surface coil for squirrel monkey spinal cord imaging. *Magn Reson Imaging*. 2020 May; 68:197-202. doi: 10.1016/j.mri.2020.02.011. Epub 2020 Feb 19. PMID: 32087231; PMCID: PMC7136156.

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

Nothing to report

- **Other Products**

Nothing to report

[7] PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

John C. Gore	No change
Nellie Byun	No change
Li Min Chen	No change
Jamie Reed	No change
Feng Wang	No change
Pai-Feng Yang	No change

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

Not applicable

What other organizations were involved as partners?

Nothing to Report.

[8]. SPECIAL REPORTING REQUIREMENTS

Not applicable

[9]. APPENDICES:

Mishra A, Wang F, Chen LM, Gore JC. Longitudinal changes in DTI parameters of specific spinal white matter tracts correlate with behavior following spinal cord injury in monkeys. *Sci Rep.* 2020 Oct 14;10(1):17316. doi: 10.1038/s41598-020-74234-2. PMID: 33057016; PMCID: PMC7560889.

Lu M, Wang F, Chen LM, Gore JC, Yan X. Optimization of a transmit/receive surface coil for squirrel monkey spinal cord imaging. *Magn Reson Imaging.* 2020 May;68:197-202. doi: 10.1016/j.mri.2020.02.011. Epub 2020 Feb 19. PMID: 32087231; PMCID: PMC7136156.

Wang F, Wu TL, Li K, Chen LM, Gore JC. Spatiotemporal trajectories of quantitative magnetization transfer measurements in injured spinal cord using simplified acquisitions. *Neuroimage Clin.* 2019;23:101921. doi: 10.1016/j.nicl.2019.101921. Epub 2019 Jul 2. PMID: 31491830; PMCID: PMC6639592.

Wang F, Zu Z, Wu R, Wu TL, Gore JC, Chen LM. MRI evaluation of regional and longitudinal changes in Z-spectra of injured spinal cord of monkeys. *Magn Reson Med.* 2018 Feb;79(2):1070-1082. doi: 10.1002/mrm.26756. Epub 2017 May 25. PMID: 28547862; PMCID: PMC5702279.

Wu TL, Yang PF, 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. *Nat Commun.* 2019 Mar 29;10(1):1416. doi: 10.1038/s41467-019-09485-3. PMID: 30926817; PMCID: PMC6440970.

Wang F, Zu Z, Wu TL, Yan X, Lu M, Yang PF, Byun NE, Gore JC and Chen LM. CEST and NOE MRI of Injured Spinal Cord in Monkeys (revision submitted to *Neuroimage Clin*)