Injury alters the intrinsic functional connectivity network in spinal cord of monkeys

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Purpose: Resting state fMRI has shown that intrinsic functional connectivity networks may be detected in the spinal cord of humans and anesthetized monkeys [1-2]. The functional relevance of these networks, however, remains to be determined. One way to address this question is to modulate the network and then correlate the changes with behavioral and functional outcomes. In this study we examined the effects of unilateral spinal cord injury on the inter-regional correlation strengths of resting state fMRI signals between spinal horns of gray matter in spinal segments above or below the injury level.

Methods: Five squirrel moneys were included in this study, two of which underwent unilateral dorsal column lesions at cervical 4-5 level. All scans were performed on a 9.4T, 21-cm bore magnet Varian INOVA MR imaging spectrometer, using a custom-designed saddle-shaped transmit-receive surface coil (2.5 × 3 cm^2 in size) positioned over the animal's neck. Five contiguous axial slices (in 3 mm thickness) were acquired in each imaging session. High-resolution (0.25 x 0.25 mm^2 in-plane resolution, 128 x 128 matrix) structural images with MTC (Magnetization Transfer Contrast, TR/TE: 220/3.24 ms) were acquired using a Gaussian RF saturation pulse (flip angle: 820°, pulse width: 12 ms, and RF offset: 5000 Hz). Functional MRI (fMRI) imaging data were collected with the same 5 - slice prescription with an in-plane resolution of 0.5 x 0.5 mm^2 (64 x 64 matrix) using a fast gradient echo sequence (flip angle: ~15°, TR: 24 ms, TE: 6.5 ms, volume acquisition time: 1.54 s). Resting state fMRI time series of 330 volumes of functional images were acquired. The functional data were slice time and motion corrected. The fMRI signal time courses were regressed with slice wise motion correction parameters along with muscle signal derived from a principal component analysis. Three major components from masked voxels in the muscle and cerebrospinal fluid regions were used as regressors, which roughly accounted for at least 70% of the cumulative variance. Seeds were identified in five regions of bilateral dorsal and ventral horns and one white matter control region. The location of the control seed was randomized across runs and animals. Each ROI typically contained 2-3 voxels. After injuries, data from slice 3 (where the lesions were placed) were excluded in the analysis. The correlations between the mean fMRI signal time courses of each paired ROI seeds were calculated. Correlation strengths among horn (ventral and dorsal, left and right) and control ROIs were compared in a pair-wise manner within each slice (intra-slice ROIs).Statistical significance was determined using a non-parametric Mann Whitney Wilcoxon test and a result of $p<0.05$ was considered as significant. The resting state analysis was performed using in house software compatible with SPM5/8.

Results: We found differential functional connectivity among intra-slice ROI horns in normal spinal cord. The strengths of the resting state correlations between different pairs of seed ROIs within each slice (intra-slice) are shown at both single animal (Fig 1a) and the group level (Fig 1e). Representative 2D matrix plots in (a) shows much stronger connectivity between horn – horn than horn – control pairs regardless of the specific slice examined. The ventral and dorsal horns on the same side exhibited the strongest connectivity (column 3). Whisker box plots of the group mean values and variations of correlation coefficients further supported this observation (compare 1-4 with 5-6 columns in Fig 1e). We next examined whether and how injury to the cord alters functional connectivity by creating unilateral lesions at C5 level (the entering zone for D2/D3 afferents). We found that within each slice, regardless of the positions above or below the lesion, the overall pattern of functional connectivity among intra-slice ROIs was similar to those in normal conditions. However, comparisons of the connectivity of ROI horns in pre- and post- lesion conditions showed significantly reduced correlation coefficients (1-4 columns in Fig 1h), indicating the intra-slice horn-horn connectivity in the below lesion slices was significantly weakened after injury. The dorsal horn – control ROI correlations were not affected. Importantly, when we divided the ventral horn pairs into the left (lesion side) and the right (intact side), and compared their correlation values between above and below lesion slices and in normal versus lesioned conditions, we found that the effects of injury were unilateral and limited to the lesion side (the left) in slices only below the injury.

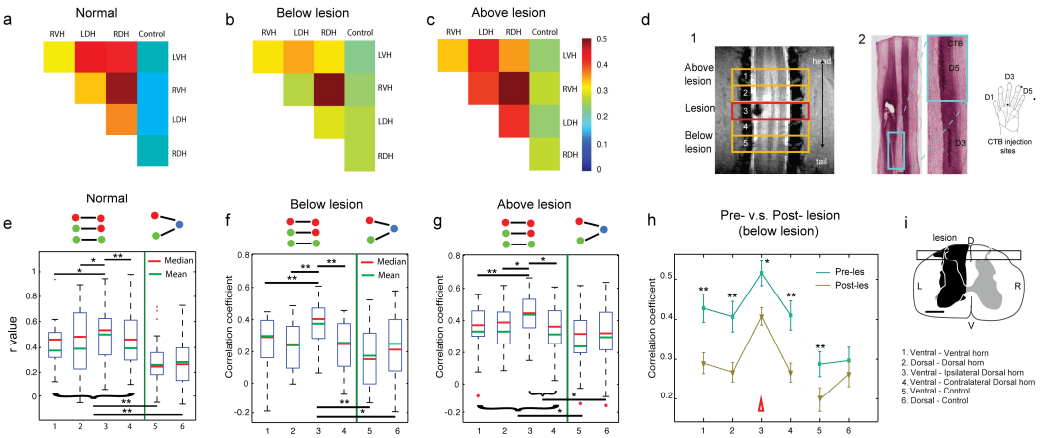

Fig 1. Differential correlation strengths among intra- slice ROIs and the effects of unilateral lesion. **a-c**. Representative 2D correlation matrix plots of the mean correlation coefficients among intra- slice ROI pairs in normal (a), below lesion (b) and above lesion (c) axial image slices. The color of the squares indicated the strength of the correlation (see color scale bar for r values). **d.** (1) Horizontal MTC image shows the actual lesion (black hole). Red rectangle outline shows the placement of the third axial image slice, which is centered at the lesion level. (2) CTB stains of the corresponding post mortem spinal cord tissue. Inserted hand shows the CTB injection sites. **e-g.** Group Whisker box car plots show the distribution of correlation coefficients across all ROI pairs in normal (e), below lesion (f) and above lesion (g) image slices. The upper and lower bounds of the box indicated the upper and lower quartile of the data**.** Red stars indicated the outlier. The red and green lines inside the blue box indicate the mean and median values, respectively. Green line separated the two ROI pairs groups: horn – horn pairs (columns 1-4) and horn – control pairs (columns 5-6). * $p<0.01$; ** $p<0.001$. **h:** Direct comparison of the mean correlation coefficients of the same set of ROI pairs obtained before (pre-lesion, green line) and after (post-lesion, yellow line) the lesions. Error bars indicated the standard deviation of the measurements. **i:** Reconstructed lesion in one representative animal. Black rectangle outline shows the location of the horizontal MRI image shown in d(1).

Discussion and conclusion: Our data show that within a spinal cord segment (sampled using a 3 mm thick slice) spinal cord gray matter horns are connected strongly to other horns, but the intra-horn connectivity varies in strength. After a unilateral lesion, the functional connectivity between horns on the lesion side and the other horns significantly reduced. The reduction of functional connectivity between ventral and dorsal horns, however, only appeared in the lesion side and in spinal segments below the injury. The quantification of resting state connectivity may thus be a useful biomarker of functional integrity in the spinal cord. Our next goal will be to examine the relationships between the reduced functional connectivity and behavioral changes in a larger sample of animals.

References: [1] Wang Z. et al. (2013): The relationship of anatomical and functional connectivity to resting-state connectivity in primate somato-sensory cortex, Neuron, 19;78(6):1116-26. [2] Mishra et al. (2014): Functional connectivity of intrinsic networks in monkey spinal cord revealed by resting state BOLD signals at 9.4T. ISMRM14, Milan: 3813.