Exploration of Functional Organization in Human Cervical Spinal Cord Using Resting State fMRI

Xiaojia Liu[1,2], Fuqing Zhou[3], Xiang Li[3], Jiaolong Cui[3], Mengye Lyu[1,2], Adrain Tsang[1,2], Iris Y Zhou[1,2], Ed X Wu[1,2], and Yong Hu[3]

[1]*Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong, Hong Kong, China,* [2]*Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong, Hong Kong, China,* [3]*Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, Hong Kong, China*

INTRODUCTION: Resting state fMRI (rsfMRI) has emerged as a powerful tool for probing the functional organization in the human brain based on spontaneous fluctuating low frequency BOLD signals [1-3]. Spinal cord, as an important part of central nervous system, plays a key role in connecting the brain and peripheral nervous system. Yet its functional organization of spinal cord is still poorly understood. In the previous studies, the distribution of functional connectivity over segments (from vertebra C3 to C5 and from vertebra C5 to vertebral T1) has been reported, which demonstrated the temporal features of the functional organization [4, 5]. However, functional connectivity only focuses on the temporal correlation which is a temporal domain characteristic of functional organization. Till now, the characteristics of functional organization in spatial and frequency domain remain unclear. In this study, we aim to explore the functional organization in the human cervical spinal cord by using graph theory analysis in spatial domain and amplitude of low-frequency fluctuation (ALFF) method in frequency domain.

METHODS: **Data acquisition & participants**: Twenty-four healthy subjects (Female/Male = 6/18, age = 30±5 years) were imaged in this study using a 3T Philips Achieva whole body MRI scanner and a 4-channel neurovascular coil. The rsfMRI images were acquired using a gradient echo EPI sequence with parameters as follows: TR/TE = 2000/30 ms, Flip Angle = 50^0, Number of Slices = 26, voxel size = 1.25x1.25x4 mm, FOV = 80x80x104 mm (from vertebrae C1 to C7). Ten of the subjects were imaged twice to perform the reproducibility test of rsfMRI evaluation on cervical cord. **Image processing**: The raw EPI images were processed using SPM8 and REST toolboxes in MATLAB for slice timing, motion correction, detrend, and band-pass filtering between 0.01 and 0.08Hz. The first 10 volumes were deleted to remove the initial transient effects. Masks were manually drawn over the gray matter on 15 out of 26 slices covering from C2 to C6 segments (3 slices for each segment) (Figure 1a). The slices crossing C1, C7 segments and intervertebral discs, were not masked due to FOV mismatch or severe artifact. Each gray matter mask was separated into ventral horn and dorsal horn (Figure 1b) due to their different involvement in neural activity (ventral horn controls motor and dorsal horn controls sensory). Thirty regions of interest (ROI) were depicted in total (5 segments, 3 slices per segment, and 2 masks per slice). Since BOLD fluctuation mainly happens in the neurons of gray matter, power spectrum density of signals inside and outside the gray matter masks were calculated to check the accuracy of ROIs placement (Figure 1c). **ROI-based functional correlation analysis**: Functional correlation was calculated using REST toolbox in MATLAB between pairs of ROIs to obtain a 30x30 correlation matrix for each subject and subsequently generate the mean correlation coefficient (CC) map. Fisher Z-transformation was performed in each correlation matrix. One-sample t-test was performed to test the correlation significance with false discovery rate (FDR) correction, based on the Z-transformed matrices from all subjects. **Graph theory analysis**: Graph theory analysis was performed using BCT toolbox in MATLAB to evaluate the communication characteristics of the functional organization in the spinal cord including global efficiency, local efficiency and small world property. Threshold was applied on the functional correlation matrix to establish binarized functional network. **Amplitude of low frequency fluctuation (ALFF) analysis**: ALFF value was calculated through REST toolbox in MATLAB to observe the neural activity distribution in the cervical cord. Two sample t-test was performed to test the significance of ALFF difference between segments. **Test retest reliability analysis**: Intra class correlation (ICC) was calculated to investigate the test-retest reliability of rsfMRI measurements on the cervical cord including network properties (global efficiency, local efficiency, small world property) and ALFF values.

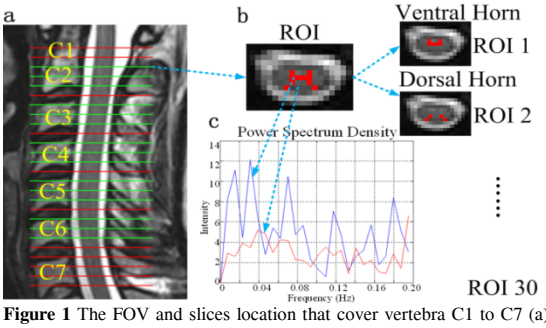

Figure 1 The FOV and slices location that cover vertebra C1 to C7 (a); ROI placement to delineate ventral horn and dorsal horn drawn on post-processed EPI images (b); power spectrum density comparison between BOLD signal inside and outside the gray matter mask (c)

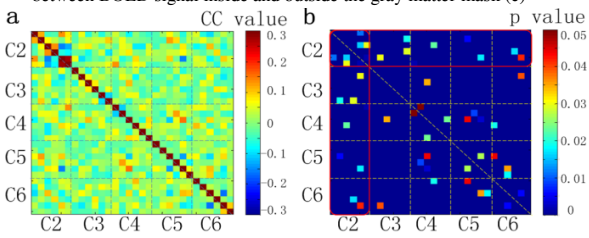

Figure 2 Mean correlation coefficient (CC) map calculated from ROI correlation analysis result from all subjects, the diagonal line is the axis of symmetry, points on diagonal line are CC values between the same ROI (a); p value map of one-sample t-test after FDR correction and points with significant correlation (p<0.05) are shown (b);

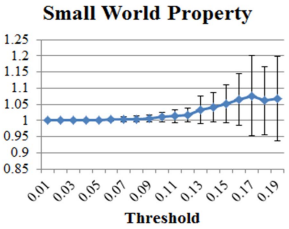

Figure 3 Small world property of the functional network under threshold from 0.01 to 0.19

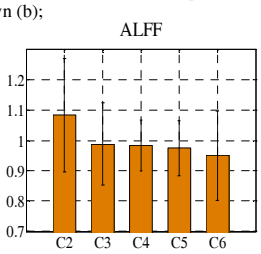

Figure 4 ALFF value distribution on the cervical spinal cord, C2 has significant higher ALFF value than other segment

Table 1 Test retest reliability

rsfMRI measurement	ICC
Global Efficiency	0.7803
Local Efficiency	0.6933
Small World Property	0.4669
ALFF	0.6048

Note: ICC ⩾ 0.4 is commonly expected in test-retest reliability examination.

RESULTS: The 30x30 symmetric matrix of mean correlation coefficient (CC) value map generated from all subjects is shown in Figure 2a. Each point represents the mean correlation coefficient between two ROIs. Figure 2b shows the p value map of the result from the one-sample t test after FDR correction, where only points with significant differences (p<0.05) are shown. Compared with other segments, C2 segment in the cervical cord have higher level of correlation. Plot of small world property under threshold from 0.01 to 0.19 was shown in Figure 3 and 0.19 is the largest threshold to maintain the integrity of the functional network in the cervical cord. The small world property is larger than one from threshold 0.03 to 0.19. Figure 4 shows the ALFF value distribution in the cervical cord and the result of two-sample t-test. C2 segment has significant higher ALFF value than other segments. The ICC results of test-retest reliability analysis are listed in Table 1.

DISCUSSION AND CONCLUSION: In this study, the correlation between ventral or dorsal horn of gray matter at different segments was investigated through ROI-based functional correlation analysis. Functional connectivity can be observed within and cross slices of spinal cord, which is consistent with previous functional connectivity studies [4, 5]. Besides, our study finds that the C2 segment showed more functional connectivity with other segments. However, functional connectivity only describes temporal characteristic of the functional organization and the spatial and frequency characteristics of functional organization are still unclear. To our best knowledge, this is the first demonstration of the spatial and frequency characteristics of functional organization in the spinal cord. In spatial domain, graph theory analysis finds that the small world property is larger than one through threshold from 0.03 to 0.19, which means the functional network model in the cervical cord can communicate efficiently in both local and global range. In frequency domain, ALFF analysis shows that C2 segment also has significant higher neural activity intensity than other segments, which is in accordance with the finding of functional correlation analysis as segment with higher level of connectivity requires more energy consumption and will result in higher neural activity intensity. Our findings suggest that the spinal cord not only is a neural pathway between brain and peripheral nervous system, but also presents some characteristics of functional network to modulate neural information. This study suggests that rsfMRI could be a robust tool to further investigate the functional organization of the spinal cord as well as the underlying pathophysiology of spinal cord in trauma and degenerative conditions.

REFERENCES: [1] Smith S M, Fox P T, Miller K L, et al. Proc Natl Acad Sci U S A., 2009, 106(31): 13040-13045. [2] Raichle M E. Brain Connect, 2011, 1(1): 3-12. [3] Fox M D, Raichle M E. Nat Rev Neurosci, 2007, 8(9): 700-711. [4] Wei, Pengxu, et al. Eur J Appl Physiol 108.2 (2010): 265-271. [5] Barry et al. eLife 2014; 3:e02812..