BOLD and Blood Volume-weighted fMRI of Rat Lumbar Spinal Cord during Non-noxious and Noxious Hindpaw Stimulation

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[INTRODUCTION] Spinal cord fMRI is a useful tool for studying spinal mechanisms of pain, hence for analgesic drug development. Even though the technical feasibility to perform spinal cord fMRI has been shown (*1-4*), several key issues need to be addressed. First, what kind of neuronal activation, noxious stimulation-induced activation or non-noxious stimulation-induced activation, or both of them, can be reliably and reproducibly detected in anesthetized rats. Second, can blood volume (BV)-weighted fMRI, which has been proven to have high sensitivity and high specificity in brain, be applied to spinal cord. Third, the spatial specificities of BOLD and BV-weighted fMRI in spinal cord are unknown. To address these issues, BOLD and BV-weighted fMRI of lumbar spinal cord due to transcutaneous noxious and non-noxious electrical stimulation of the hindpaw were performed in α-chloralose anesthetized rats.

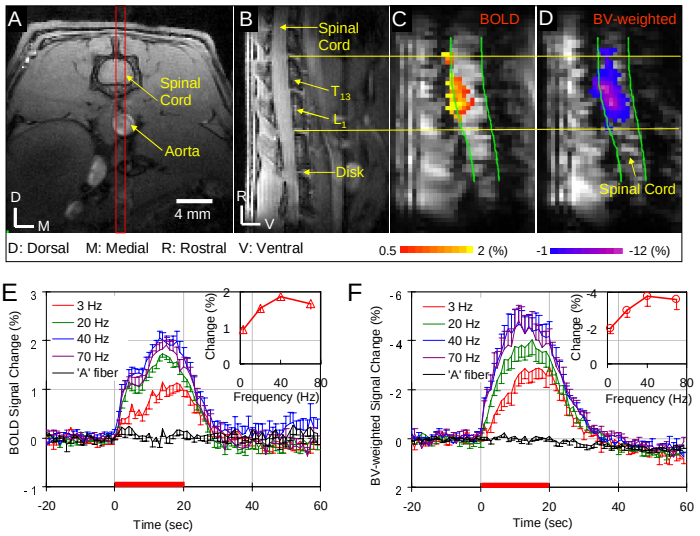

Fig. 1 BOLD and BV-weighted fMRI of lumbar spinal cord with right hindpaw stimulation. The axial image (A) show the localization of the sagittal image in which the anatomical image (B) and fMRI (C and D) were acquired (outlined by red lines). Vertebrae level can be identified in (B) and referenced to (C and D). Noxious electrical stimulation-induced BOLD (C) and BV-weighted fMRI data (D) for a single rat are displayed as relative signal changes. For both BOLD and BV-weighted fMRI, activations were mainly observed in the dorsal region of the cord. fMRI changes locate in the spinal cord segments of L3 - L5. Across animal averaged time courses of BOLD (E) and BV-weighted fMRI (F) in response to non-noxious stimulation (black lines) and noxious stimulation of different frequencies (mean ± SEM, n=6). Red bars under the time courses indicate the 20-s stimulation period (E and F). The insets show the response amplitude versus the stimulus frequency at the condition of noxious stimulation.

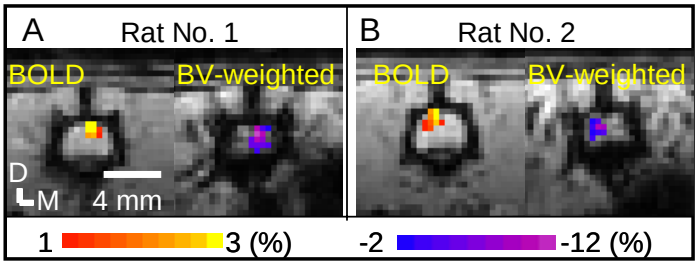

Fig. 2. BOLD and BV-weighted fMRI in axial direction from two animals. Peak activations of BOLD responses (red/yellow) are located in and near the surface of the ipsilateral dorsal horn, while peak activations of spinal blood volume responses are localized to the middle of the ipsilateral dorsal horn. Note that (A) is from right hindpaw stimulation while (B) is from left hindpaw stimulation. D: Dorsal; M: Medial.

[METHODS] The animal protocol was approved by the IACUC of Merck Research Laboratories. All MRI measurements (n=7) were performed on a 4.7T Bruker Biospec system. A 2 cm diameter surface coil positioned beneath the lumbar spinal cord of the rat was used as the RF receiver, while an actively-decoupled 72-mm diameter volume coil was used as the RF transmitter. Anatomical images in two directions (axial and sagittal) were first acquired by a FLASH sequence. From the sagittal image, the 13th rib was identified and used as a landmark to locate the appropriate vertebral levels. T2*-weighted images were acquired using a single-shot GE EPI with phase-encoding in the dorsal-ventral direction; matrix size = 64 × 64; TE=12.4 ms; FOV = 4 cm (rostral-caudal direction) × 2 cm (dorsal-ventral direction). Each run consisted of 20-**20**-40 image acquisitions (boldface represents stimulation on) with TR=1 sec. To differentiate the innocuous and nociceptive responses in the spinal cord, non-noxious stimuli (0.3ms, 1.5mA) were chosen to activate A-fibers and noxious stimuli (2ms, 5mA) were chosen to activate C-fibers (5). To investigate the stimulation frequency dependence of the BOLD and BV-weighted fMRI signals to noxious stimulation, noxious stimuli with varying frequencies of 3, 20, 40, and 70 Hz were investigated. To examine the locations of BOLD and BV-weighted fMRI signals in the cross-sectional direction of the spinal cord by unilateral hindpaw stimulation, multiple-slice axial images were further acquired in vertebral levels of T13 and L1. Matrix size = 64 × 64, field of view = 2.5 × 2.5 cm^2. t-test was used to detect the activation. BOLD and BV-weighted fMRI data were acquired before and after injection of USPIO (15mg Fe/kg), respectively.

[RESULTS & DISCUSSION] No activations were observed with non-noxious stimuli for either BOLD or BV-weighted fMRI. Noxious stimuli elicited robust ipsilateral activations in spinal cord for both BOLD and BV-weighted fMRI in all rats and, the activation foci for each rat were similar under different stimulation frequencies. Fig. 1C (BOLD) and Fig. 1D (BV-weighted) show the activation maps from a representative rat. In BOLD fMRI, the highest percentage signal changes (yellow pixels) were mainly seen near the spinal cord dorsal surface (green contours). In BV-weighted fMRI, the areas of highest signal change (violet pixels) locates in the middle of the ipsilateral dorsal horn, which roughly corresponds to laminae V and VI. Time courses of BOLD (Fig. 1E) and BV-weighted fMRI (Fig. 1F) signals under different stimulation conditions were obtained from the same ROI, respectively. Non-noxious stimulation did not induce an observable signal change (black lines in Figs 1E and 1F). For noxious stimulation, the temporal profiles of BOLD and BV responses did not vary with the stimulus frequency. The response amplitudes varied significantly with the frequency of the noxious stimulation. In BOLD fMRI, the highest change (1.87 ± 0.27%, n=6) was detected at 40 Hz. In BV-weighted fMRI, the highest change (-3.77 ± 1.32%, n=6) was also detected at 40 Hz. To compare the sensitivity of two different fMRI techniques, CNRs with 40 Hz noxious stimuli were determined within the ROI used for the above quantitative analysis. CNRs were 0.63 ± 0.14 and 0.86 ± 0.24 (n = 6 animals) for BOLD and BV-weighted fMRI, respectively. To determine reproducibility, fMRI data by noxious stimulation were divided into two subsets, even and odd runs and activation maps were determined individually from the subsets. Reproducibility of the fMRI signal changes within the ROI was determined with linear correlation analysis. For BOLD fMRI the averaged cross correlation coefficient between the two maps within the ROI for all animals was 0.73 ± 0.12 (n=6, all $p < 5.1 \times 10^{-6}$); for BV-weighted fMRI, the average CCC between the two maps within the ROI for all animals was 0.89 ± 0.05 (n=6, all $p < 3.2 \times 10^{-15}$), indicating that both BOLD and BV-weighted fMRI of spinal cord to noxious stimulation are highly reproducible. Figs. 2A and 2B show the activation maps in one axial slice from two different rats. As expected, the activation was in the ipsilateral side of the stimulated hindpaw. Consistent with studies in the sagittal direction, the highest BOLD signal change locates in the spinal cord surface, while the highest BV change locates in the middle of the dorsal horn. This study demonstrates that spinal cord fMRI can be performed in anesthetized rats reliably and reproducibly offering it as a potential tool for analgesic drug discovery.

[Reference] 1. Lilja *et al.*, *J Neurosci* 26, 6330 (2007). 2. Yoshizawa et al., *Neuroimag* 4, 174 (1996). 3. Stroman et al., *MRI* 19, 827 (2001). 4. Majcher *et al.*, *NeuroImage* In Press, (2007). 5. Le Bars et al., *Pain* 6, 283 (1979).