

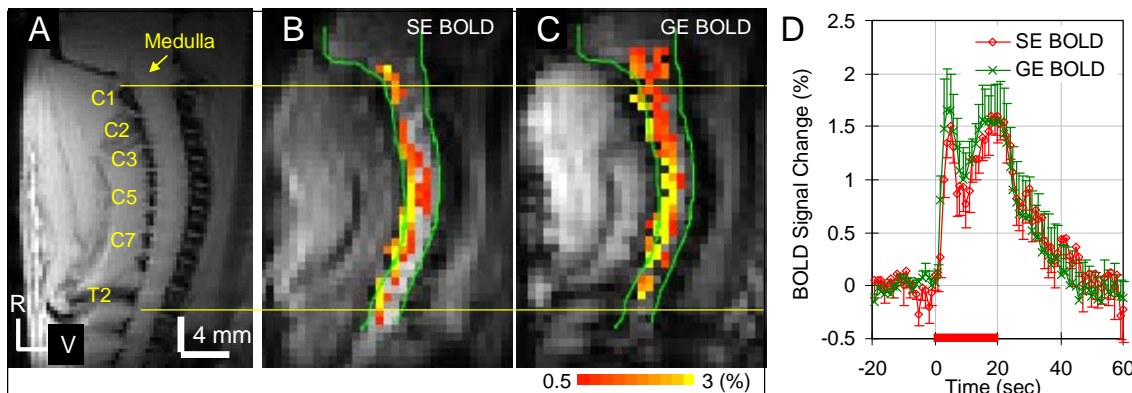
# Gradient-echo and spin-echo BOLD fMRI of rat spinal cord – insight into its hemodynamic response to neuronal activity

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**[INTRODUCTION]** Spinal cord fMRI has been of interest for a long time, but the mechanisms of hemodynamic response in spinal cord to neuronal activity have not been clearly elucidated. Whether the hemodynamic response in spinal cord is similar to that in brain is unknown. In this study, spin-echo and gradient-echo BOLD fMRI were performed in the rat cervical spinal cord to answer this question. Based on analytical biophysical models (1, 2) and Monte Carlo simulations (3, 4), a change in GE BOLD fMRI signal is sensitive to blood oxygen level change occurring in vessels of all sizes and a change in SE BOLD fMRI signal is heavily weighted to the microvasculature. Based on different vessel size dependence of SE and GE BOLD signals, by comparing the SE and GE BOLD measured by EPI, the vasculature involved in BOLD fMRI response in the cortex has been investigated (5-9). Performing GE and SE BOLD measurements in the same animal under identical stimulation conditions in spinal cord will allow the spinal cord fMRI results to be quantitatively compared with those of brain, and will provide insights into hemodynamic response in spinal cord. This study concludes that the hemodynamic response and the vasculatures involved in BOLD fMRI of spinal cord are similar to those of cerebral cortex.

**[METHODS]** The animal protocol was approved by the IACUC of Merck Research Laboratories. Following an initial 60-mg/kg bolus of  $\alpha$ -chloralose, the rat was maintained on a continuous infusion of 30 mg/kg/hr  $\alpha$ -chloralose and 4 mg/kg/hr pancuronium bromide. All MRI measurements were performed on a 7T Bruker Biospec system. A 3 cm diameter surface coil positioned on the top of the cervical 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. T2\*-weighted images were acquired using a single-shot GE and SE EPI with phase-encoding in the dorsal-ventral direction; matrix size =  $64 \times 64$ ; FOV = 6 cm (rostral-caudal direction)  $\times$  4 cm (dorsal-ventral direction); gradient echo time (TE) = 11 ms, spin echo time (TE) = 35 ms. Each run consisted of 20-20-40 image acquisitions (boldface represents stimulation on) with TR=1 sec. To achieve the highest activation in spinal cord, a optimum electrical pulse strain (2ms, 5mA, 40Hz) (10) was applied to the bilateral forepaws simultaneously. One single sagittal slice of 2 mm thickness covering the bilateral dorsal horns was chosen. Signals from all fMRI runs for each animal under the same conditions were averaged. Averaged data were processed using Stimulate and MATLAB routines (Mathworks, Natick, MA). Statistical t value maps were computed by comparing the experimental fMRI data acquired during control and stimulation periods on a pixel-by-pixel basis. For quantitative analyses of fMRI activation, a common region of interest (ROI) was used for GE and SE BOLD fMRI in each animal.



**Figure** (A) The anatomical sagittal image corresponding to the fMRI slice which was acquired by FLASH sequence with the exact same field of view as the fMRI study. The vertebrae levels can be easily identified. Noxious stimulation-induced SE BOLD (B) and GE BOLD (C) are displayed as relative signal changes for voxels that exceeded the statistical threshold ( $p < 0.05$  and cluster size of 4) within the spinal cord. Green lines outline the surface of spinal cord in (B) and overlaid on (C). Two horizontal yellow lines indicate the positions of rostral disk of C1 and caudal disk of T2. R: Rostral; V: Ventral. (D) shows averaged time courses of GE BOLD and SE BOLD (mean  $\pm$  SEM,  $n=5$ ). Red bars under the time courses indicate the 20-s stimulation period.

## [RESULTS & DISCUSSION]

Robust activations were detected in cervical spinal cord in all rats. Fig. B-C shows the activation maps of SE and GE BOLD fMRI from one rat. In dorsal-ventral direction, activations of both GE and SE BOLD are mainly located in the dorsal region of the spinal cord, consistent with the neuroanatomy of synapse location of somatic peripheral sensory fibers. For GE BOLD (Fig. C) the highest signal changes (yellow pixels) were mainly seen near the spinal cord dorsal surface (green contours). This observation is consistent with previous GE BOLD studies of rat lumbar spinal cord (10), rat

somatosensory cortex, and cat visual cortex, where the highest BOLD signal changes were seen in lumbar spinal surface and cortical surfaces due to the large draining vein. For SE BOLD (Fig. B), the highest signal change was also mainly seen near the dorsal surface. In rostral-caudal direction, the highest activations occur between the vertebrae level C4 and C7, roughly matching the location of the afferent terminals from the median and ulnar nerves which originate from the plantar side of the forepaw. Time courses of SE and GE BOLD fMRI signals were obtained from the same common ROI. A notable feature is that both GE and SE BOLD time courses show humps at the initial response. Such humps are caused by arterial blood pressure increase due to an autonomic response to noxious stimulation, and has been verified with simultaneous BP recordings (data not shown). The percent signal change within the ROI for GE BOLD fMRI is  $1.37 \pm 0.60$ , for SE BOLD fMRI is  $1.24 \pm 0.35$ , ( $n=5$  animals). Since the stimulation-induced relaxation rate change for GE BOLD ( $\Delta R2^*$ ) does not depend on the vessel size, while the stimulation-induced relaxation rate change for SE BOLD ( $\Delta R2$ ) depend on the vessel size, the ratio of  $\Delta R2^*$  to  $\Delta R2$  is related to the size of vessels responding to BOLD fMRI (4).  $\Delta R2^*$  and  $\Delta R2$  can be estimated from the percentage signal changes as  $(\Delta S/S)/TE$  for both GE BOLD and SE BOLD under the assumption that the intravascular contribution is minimal at high magnetic fields because of shorter T2\* ( $> 4.7$  T). In this study, relative GE-EPI fMRI signal change with TE of 11 ms is 1.37%, yielding a stimulation-induced relaxation rate change  $\Delta R2^*$  of  $0.0137 / 0.011 = 1.25 \text{ s}^{-1}$ ; the relative SE fMRI signal change with TE of 35 ms is 1.24%, yielding a stimulation-induced relaxation rate change  $\Delta R2$  is  $0.0124 / 0.035 = 0.35 \text{ s}^{-1}$ .  $\Delta R2^* / \Delta R2$  of cervical spinal cord in this study is  $1.25 / 0.35 = 3.52$ , which is consistent with the ratios of 3.3-4.0 observed in various human cerebral cortical regions (5-7), 3.3 in rat somatosensory cortex (8) and 3.3 in cat visual cortex (9), at magnetic fields ranging from 1.5 T to 9.4 T. The similarity of these ratios between spinal cord and cortex provides additional support to the concept that the hemodynamic response and the vasculature of the spinal cord are similar to that of cerebral cortex.

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