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Pain fMRI in rat cervical spinal cord: An echo planar imaging evaluation of sensitivity of BOLD and blood volume-weighted fMRI

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ABSTRACT

Objective measure of pain is valuable in drug discovery research and development of analgesics. Spinal cord is an important relay of the pain pathway, and fMRI offers an excellent opportunity to quantify pain using activation in the spinal cord induced by painful stimuli. fMRI literature of cervical spinal cord with regard to the spatial extent, in both longitudinal and cross-sectional directions, of neuronal activation induced by noxious stimulation is ambiguous. This study investigates the feasibility of developing a robust pain assay using fMRI in the cervical spinal cord in α-chloralose anesthetized rats subjected to transcutaneous noxious electrical stimulation of the forepaw. Blood oxygenation level dependent (BOLD) and blood volume (BV)-weighted fMRI data were acquired without and with intravenous injection of ultra small superparamagnetic iron oxide particles (USPIO), respectively. BOLD data were acquired by gradient-echo (GE) and spin-echo (SE) echo planar imaging (EPI), while BV-weighted fMRI data were acquired only by GE EPI. Cervical spinal cord activity was robustly detected by all three fMRI techniques. The sensitivity of the fMRI signal was highest in GE BV-weighted fMRI followed in order by GE BOLD, and SE BOLD, respectively. Spatially, the fMRI signal extended ~9 mm in the longitudinal direction, covering C_4–C_8 segments, coinciding with the synapse location of afferent terminals from the stimulated site. In the cross-sectional direction, the signal change is localized predominantly to the ipsilateral dorsal region. This study demonstrates that cervical spinal cord fMRI can be performed reliably in anesthetized rats offering it as a potential tool for analgesic drug development.

Introduction

Objective measure of pain is valuable in discovery of analgesics to discriminate pain perception from psychological confounding factors and to provide information on mechanisms of action. Spinal cord is an important relay of the pain pathway, and measuring neuronal activation elicited by painful stimuli has been of interest for several decades (Kolmodin and Skoglund, 1960; Menetrey et al., 1977; Wall, 1960). Functional magnetic resonance imaging (fMRI) can detect the neuronal activations non-invasively in central nervous system with spatial resolution in sub-millimeter scale and temporal resolution in sub-second scale. Since its introduction in 1990s (Bandettini et al., 1992; Belliveau et al., 1990; Kwong et al., 1992; Ogawa et al., 1990, 1992), it has been widely used to study brain function in relation to pain (Peyron et al., 2000). Even though fMRI of cervical spinal cord has been extensively pursued in both human and animals (Backes et al., 2001; Brooks et al., 2008; Lawrence et al., 2007; Madi et al., 2001; Majcher et al., 2006; Malisza and Stroman, 2002; Stroman et al., 2005; Yoshizawa et al., 1996), results regarding the spatial extent of the detected activation in both longitudinal and cross-sectional directions are ambiguous and not entirely consistent across different methodologies. In the longitudinal direction, previous fMRI studies are limited to transverse slices covering only a part of the cervical spinal cord, and the true longitudinal extent of activation coverage is not conclusive. In the cross-sectional direction, previous fMRI studies show that activations induced by ipsilateral forepaw stimulation locate not only to ipsilateral dorsal region, but also to the contralateral ventral region and areas around the center canal, in conflict with the results from electrophysiology and radiolabeled 2-deoxyglucose (2DG) techniques (Kadekaro et al., 1985; Menetrey et al., 1977).

Neuronal activity leads to a series of physiological events, including localized increases in blood flow, blood volume, and metabolic rate of oxygen, which are collectively termed “the hemodynamic response”. Such a hemodynamic response causes an alteration in venous blood oxygenation level, which can be detected by the blood oxygen level dependent (BOLD) fMRI technique (Ogawa et al., 1990, 1993). BOLD fMRI data can be acquired by either a gradient-echo (GE) approach, or a spin-echo (SE) approach. Based on analytical biophysical models

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(Kiselev and Posse, 1999; Yablonskiy and Haacke, 1994) and Monte Carlo simulations (Boxerman et al., 1995; Ogawa et al., 1993), a change in GE BOLD signal is sensitive to blood oxygen level change occurring in vessels of all sizes and a change in SE BOLD signal is heavily weighted to the microvasculature. Even with this difference in the physiological source of the fMRI signal, in studies reported in humans and animals at spatial resolutions of millimeter to sub-millimeter scale, the anatomical locations of activation detected by GE and SE approaches are the same (Yacoub et al., 2005; Zhao et al., 2004, 2006). Performing GE and SE measurements in the same animal under identical stimulation conditions allows the evaluation of the robustness of the fMRI technique for detection of neuronal activation in the cervical spinal cord. Furthermore, cervical spinal cord fMRI was pursued previously mainly by fast spin-echo sequence (e.g., RARE, rapid acquisition with relaxation enhancement) (Lawrence et al., 2007; Stroman et al., 2005), while echo planar imaging (EPI)-based GE and SE BOLD have been widely used for brain fMRI (Bandettini et al., 1993; Kwong, 1995). 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 (Bandettini et al., 1994; Jones et al., 1998; Lee et al., 2002; Lowe et al., 2000; Zhao et al., 2004). Hence, performing GE EPI and SE EPI fMRI in cervical spinal cord will allow the results to be quantitatively compared with those of brain, and will provide insights into hemodynamic response in spinal cord.

In addition to BOLD fMRI, neuronal activation can also be detected by blood volume (BV)-weighted fMRI technique using an intravascular contrast agent such as ultra-small superparamagnetic iron oxide particles (USPIO) or monocrystalline iron oxide nanoparticles (MION) (Kennan et al., 1994; Leite et al., 2002; Lu et al., 2007; Mandeville et al., 1998; van Bruggen et al., 1998; Zhao et al., 2006), which also is referred to as IRON technique, Increased Relaxation for Optimized Neuroimaging (Leite and Mandeville, 2005). It has been proved that the BV-weighted fMRI by GE EPI has high sensitivity in both brain and lumbar spinal cord (Zhao et al., 2006, 2008). By performing GE BV-weighted, SE and GE BOLD fMRI in the same rats, feasibility of cervical spinal cord fMRI by these different techniques can be further ascertained. Most importantly, since the SE EPI has not been used in the spinal cord fMRI yet, the utility of SE EPI in the spinal cord fMRI can be verified.

The purpose of this study is two-fold: 1) to investigate the spatial extent of the detected activation in both longitudinal and cross-sectional directions of cervical spinal cord induced by noxious electrical stimulation of the forepaws by using BOLD and BV-weighted fMRI; 2) to demonstrate the utility of SE EPI as a potential tool for spinal cord fMRI studies. BOLD fMRI was performed by GE EPI and SE EPI, while BV-weighted fMRI was performed by GE EPI, in the cervical spinal cord of α-chloralose anesthetized rats at 7 T. Our previous data (Zhao et al., 2008) demonstrated that the noxious stimulation (using electrical pulses with 2 ms duration, 5 mA amplitude, and 40 Hz frequency) of hindpaw induced the maximum hemodynamic response in lumbar spinal cord, and that this signal can be robustly detected by BOLD and BV-weighted fMRI. In this study, we applied such stimulation to the rat forepaw. After BOLD data were acquired, the animals were administered USPIO and BV-weighted fMRI data were acquired. fMRI data of a single-2 mm sagittal slice was acquired to view the extent of activation in the rostral–caudal direction. This slice covered the bilateral dorsal horns cross-sectionally and the region from brainstem to thoracic ~T_3 longitudinally. The maximum activation induced by bilateral forepaw stimulation is expected to be detected in this slice because peripheral nerves from the rat forepaw are known to synapse to the dorsal horn of the spinal cord. To examine the locations of BOLD and BV-weighted fMRI signals in the cross-sectional direction of the spinal cord, multiple-slice axial images were further acquired in vertebral levels of ~C_2 and ~T_1.

Materials and methods

Animal preparation and stimulation

The animal protocol was approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories. Five male Wistar rats weighing 240 to 310 g (Taconic Farms Inc, Hudson, NY, USA) were studied. Animals were initially anesthetized with 5% isofluorane in a 1:2 mixture of O_2:N_2 gases and intubated for mechanical ventilation (MRI-1; Kent Scientific, CT, USA). The respiration rate was set at 60 breaths/min. The isofluorane level was reduced to 2% and two tail veins were catheterized to deliver α-chloralose and contrast agent, respectively. After catheterization, isofluorane anesthesia was switched to α-chloralose. Following an initial 60-mg/kg bolus of α-chloralose, the animal was maintained on a continuous infusion of 30 mg/kg/hr α-chloralose and 4 mg/kg/hr pancuronium bromide. The animal was carefully secured in the prone position in a Bruker rat cradle. Before fMRI data acquisition, at least 1 h was allowed for physiological stabilization of the animal and clearance of isofluorane, during which anatomical images were acquired. Body temperature was measured by a rectal probe and maintained at 37±0.5 °C during all measurements by means of a temperature-controlled air heater (SA Instruments, Inc., Stony Brook, NY USA). For the BV-weighted fMRI, in-house synthesized USPIO as described in the previous paper (Zhao et al., 2008) was used at a dose of 15 mg/kg.

Forepaw electrical stimulation was applied either bilaterally (fMRI in the sagittal slice) or unilaterally (fMRI in axial slices) using needle electrodes inserted under the skin in plantar side and connected to a constant current stimulation isolator (A365D, World Precision Instruments, Inc., Sarasota, FL, USA), which was triggered by a pulse generator (Master 8, AMPI, Israel). To assure correct placement of the electrodes, a short sequence of pulses (0.3 ms, 2 mA, 9 Hz) was applied before placing the animal in the magnet to make sure that digits twitches could be observed. A noxious stimulus (2 ms, 5 mA, 40 Hz), which is known to maximally evoke the C-fiber response based on electrophysiology and fMRI studies (Le Bars et al., 1979; Zhao et al., 2008), was used to investigate the fMRI responses in the cervical spinal cord.

General MRI experiments

All MRI measurements were performed on a 7 T, 30-cm bore Bruker AVANCE-DBX system. The gradient coil used was an actively shielded 12-cm inner diameter set with a maximum gradient strength of 40 G/cm and a rise time of 88 μs. An actively-decoupled 3 cm diameter surface coil positioned above the cervical spinal cord of the rat was used as the radiofrequency (RF) receiver, while an actively-decoupled 72-mm diameter volume coil was used as the RF transmitter. The following procedure was used to locate the region of spinal cord for fMRI acquisition. Anatomical images in three directions (axial, coronal and sagittal) were acquired by a FLASH sequence. From the sagittal image, the 2nd thoracic spinous process was identified and used as a landmark to locate the appropriate vertebral levels (see Fig. 1C). The spinal cord segments between the first cervical (C_1) and second thoracic (T_2) vertebrae were positioned in the iso-center of the magnet, and magnetic field homogeneity was optimized by automatic global shimming. Anatomical images in three directions (axial, coronal and sagittal) by FLASH sequence were then acquired for fMRI slice selection. Each fMRI run consisted of a period of 8 s dummy scans without data acquisition to achieve a steady state, followed successively by the periods of non-stimulation (20 s), stimulation (20 s), and non-stimulation (40 s), during which images for BOLD or BV-weighted fMRI data were continuously acquired. After each fMRI run, image reconstruction and data storage were performed. The total time for one fMRI run is ~2 min, including resonance frequency setting, data acquisition, image reconstruction, and data storage. The inter-