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Short communication

Diffusion tensor imaging of mouse brain stem and cervical spinal cord

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ABSTRACT

In vivo diffusion tensor imaging measurements of the mouse brain stem and cervical spinal cord are presented. Utilizing actively decoupled transmit/receive coils, high resolution diffusion images (117 μm × 59 μm × 500 μm) were acquired at 4.7 T within an hour. Both brain stem and cervical spine displayed clear gray-white matter contrast. The cervical spinal cord white matter showed similar tissue characteristics as seen in the thoracic cord. The coherent fiber orientation in the white matter was observed in both the brain stem and the cervical spinal cord. The results may serve as a reference for future inter-lab comparison in mouse brain stem and cervical spine diffusion measurements.

1. Introduction

Diffusion tensor imaging (DTI) is one of the most versatile magnetic resonance imaging (MRI) modalities for longitudinal evaluations of central nervous system (CNS) disorders in rodent models of human diseases and in patients (Dong et al., 2004; Le Bihan et al., 2001). Recently, *in vivo* DTI derived $\lambda_{\parallel}$ and $\lambda_{\perp}$ have been shown to correlate well with axon and myelin integrity, respectively (Kim et al., 2006). Findings from animal models suggest that *in vivo* DTI acquisition is well suited to detect CNS white matter injury. Most preclinical rodent DTI studies have been conducted on brains since respiratory and cardiac motions are less of a concern. Efforts to overcome motion artifacts and characterize diffusion properties in the spinal cord using DTI have been reported in rats (Ellingson et al., 2008; Franconi et al., 2000; Gullapalli et al., 2006; Madi et al., 2005).

In the use of rodents for preclinical studies, mouse models of human diseases have been widely employed because of its low cost, easy handling, and the readily achieved genetic manipulation. Numerous experimental mouse models have been developed for CNS disorders including multiple sclerosis (Cross et al., 1993), amyotrophic lateral sclerosis (ALS) (Bruijn et al., 2004), traumatic spinal cord injury (Kim et al., 2007a), etc. Studies have been performed with transgenic, and wild type mice to investigate the underlying mechanism responsible for the neurological deficit and to pursue the effective therapy (Halder et al., 2007). Damages to the cervical spine are commonly seen in multiple sclerosis, traumatic spinal cord injury, and neurodegenerative diseases. For example, motor neuron degeneration from cortex to brain stem with reported spinal cord lesions has been reported in ALS (Bruijn et al., 2004). As mouse models of human CNS lesions are disseminated temporally and spatially mimicking human clinical cases, a noninvasive assessment of the axonal and neuronal change in cervical cord and the upper and lower brain is critical to understand the fundamental morphological and pathophysiological changes.

Compared to rats (Ellingson et al., 2008; Franconi et al., 2000; Gullapalli et al., 2006; Madi et al., 2005), *in vivo* DTI observations of mouse spinal cord have been less common with only ex vivo (Niessen et al., 2006; Ong et al., 2008) and *in vivo* observations in mid thoracic spinal cords (Bilgen et al., 2005; Bonny et al., 2004; Kim et al., 2007a,b). In this study, we present *in vivo* DTI maps of the mouse brain stem and cervical spinal cord at 4.7 T using the actively decoupled RF transmitter and receiver. The pixel based *in vivo* DTI parameters including diffusion anisotropy, directional diffusivities, principal eigenvector, and diffusion ellipsoid are reported to reflect tissue structures.

2. Materials and methods

2.1. In vivo DTI

Five 10-week-old female C57BL/6 mice were anesthetized using isoflurane/oxygen mixture (7% for induction and 0.7–1.5% for

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maintenance) with warm water circulating in a pad for maintaining body temperature at 37 °C. A customized stereotaxic brain holder with ear bar and nose cone was employed to hold the mouse in a prone position allowing the spine holder, similar to that described previously (Kim et al., 2007a), to immobilize the cervical spine as well as position the surface receive coil. The nose cone was used to deliver isoflurane/oxygen mixture and to detect the change of inline pressure during the respiratory cycle. The detected inline pressure change was detected using a pressure transducer and converted to a TTL signal for synchronization of MR data acquisition with animal's respiratory motion. The gated acquisition device as employed has been reported previously (Kim et al., 2007a).

Actively detuned two-coil system was employed for DTI data acquisition. Improved coil performance through active detuning of MR coils with pin diode has been reported previously (Barberi et al., 2000; Mellor and Checkley, 1995; Vaughan et al., 2002). The simplified circuit design of coils used in this study is shown in Fig. 1a. The volume RF transmit coil is 6 cm in diameter and 10 cm long. Two 1–30 pF tunable capacitors (Voltronics, NJ) for matching and tuning along with a 4.7-pF chip capacitors (American Technical Ceramics, New York) were used in the RF transmitter. Two 0.5–10 pF ceramic tunable capacitors (Voltronics, NJ) were used to control matching and tuning of the surface receive coil. The shape of the surface coil was fit to the posterior quarter of the mouse brain and the cervical spine combining circular shape with 1.5 cm diameter covering the brain stem with the saddle shape legs of 1 cm in length to cover the cervical spine (Fig. 1a).

The entire preparation was placed in an Oxford Instruments magnet (4.7 T, 40-cm clear bore) equipped with a 10-cm inner diameter, actively shielded Magnex gradient coil (up to 60 G/cm, 200 μs rise time). The magnet, gradient coil, and gradient power supply were interfaced with a Varian NMR systems (Palo Alto, CA) UNITY INOVA console controlled by a Sun Blade 1500 workstation (Sun Microsystems, Santa Clara, CA).

The anatomical image (Fig. 1b) was acquired using a respiratory gated conventional spin-echo imaging sequence: repetition time (TR) 1.2 s (modulated by the respiratory rate), spin-echo time (TE) 10 ms, slice thickness 0.5 mm, field of view 1.5 cm × 1.5 cm, data matrix 128 × 256 (phase encoding × readout), zero filled to 256 × 256.

Diffusion-weighted images were acquired using a conventional spin-echo imaging sequence modified by adding Stejskal-Tanner diffusion weighting gradients (Stejskal, 1965) without motion compensation. The acquisition parameters were: repetition time 1.2 s (slightly varied with the respiratory rate), spin-echo time 36 ms, time between application of gradient pulses (Δ) 21 ms, diffusion gradient duration (δ) 6 ms, slice thickness 0.5 mm, diffusion sensitizing gradients orientations: (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (−1,1,0), (0,−1,1), and (1,0,−1). Based on our previous experience on *in vivo* DTI of mouse spine, the *b* value of 1.414 ms/μm^2 resulted in the best diffusion results in mouse spine. Ten transverse images of brain stem and cervical spinal cord were planned in horizontal scout image centered at C1 spinal cord (Fig. 1b) with 1.0 mm gap covering from brain stem to C5 cervical spinal cord with the

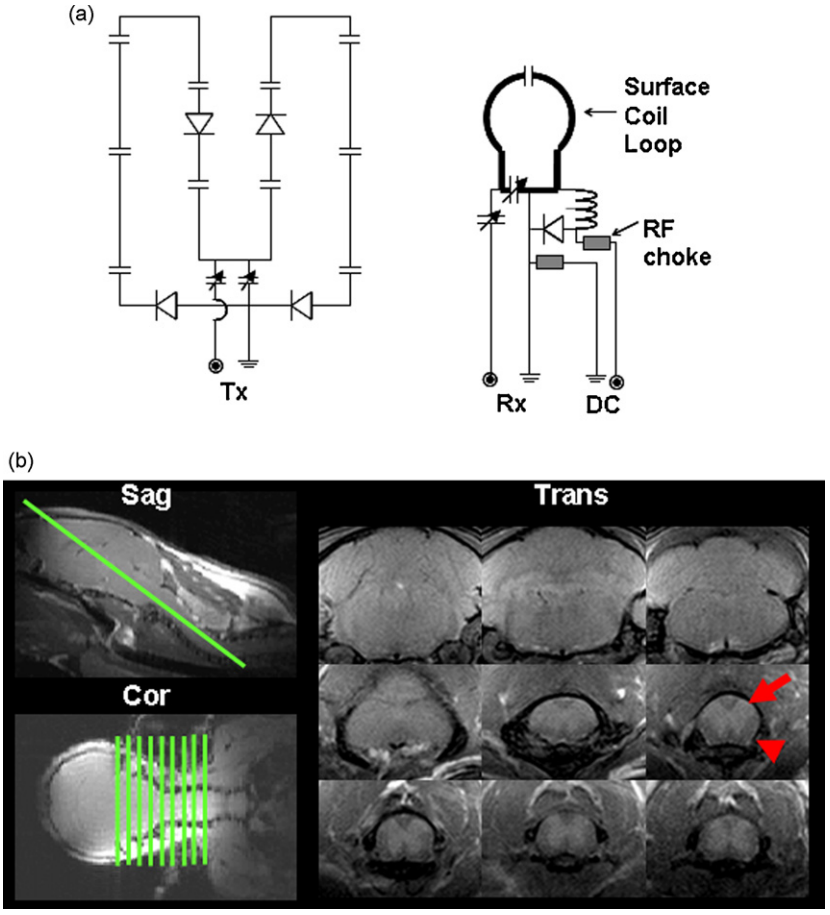

Fig. 1. The circuit diagram of actively decoupled RF transmit (left) and receive (right) coils are demonstrated in panel (a). The isolation between the two coils is achieved by the application of +5 V DC to the receive coil during RF excitation to the PIN diode (Voltronics, NJ). The RF choke (Coilcraft, IL) is used to prevent noise breakthrough. The coil loop reflects the actual shape of the receive coil conforming to the anatomy of brain stem and cervical spine (b). The transverse images are centered at C1 segment of the spine. Each image is 0.5-mm thick with 1.0-mm gap between images. The typical gray (bright, the arrows) and white (dark, the arrow heads) matter contrast is seen.