Laminar specific MEMRI enhancement of the rat spinal cord in vivo

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Introduction:

MRI of spinal cord architecture *in-vivo* is important for a number of diseases. However, it is not yet possible to visualize specific sensory and motor pathways within spinal chord grey matter due the high resolution and contrast required. Although high resolution spinal cord imaging can be obtained *in-vitro* (1), *in-vivo* imaging of the spinal cord remains a challenge due to several factors such as the susceptibility to motion, variation in tissue depth over the length of the cord, and susceptibility artifact from the cerebrospinal fluid (CSF) and vertebrae. Furthermore, it is not clear that MRI can give contrast related to spinal chord layers.

Manganese Enhanced MRI (MEMRI) utilizing different manganese chloride ($MnCl_2$) delivery methods, has yielded architectural, functional and connection information about brain areas (2,3). As in the brain, the spinal cord grey matter could be divided into different laminae, where each of the laminae receives input from a specific type of neuronal population and process or serves as a relay region in a specific sensory or motor pathway. This type of laminar arrangement in the spinal cord is currently only visualized by histological methods. It is of significant interest to determine whether laminar specific enhancement by Mn^{2+} can be achieved in the spinal cord, as has been reported in the brain and olfactory pathway (3). Major factors in applying this technique in the spinal cord are the ability to acquire high resolution spinal cord images and to determine a noninvasive route of administration which will result in uptake by the CNS.

We have applied the MEMRI approach by intravenous delivery of $MnCl_2$ and imaged lumbar spinal cord levels in rats to determine whether T_1 weighted MRI can detect spinal chord layers 48 hours following $MnCl_2$ administration. T_1 weighted spin echo images of the lower lumbar level were obtained from $MnCl_2$ injected and control rats. Here we demonstrate layer specific signal enhancement in the spinal cord of rats administered with $MnCl_2$ vs. controls in MRI of the chord with 69 micron in-plane resolution. The regions with the largest T_1 enhancements were observed to correspond to layers that contain either high cell density or large motorneurons, making MEMRI an emerging tool for studying spinal cord architecture and physiology in different animal models.

Methods:

$MnCl_2$ administration: Animal procedures were performed in accordance with NIH animal care and use guidelines. Male Sprague-Dawley rats (200g) were anesthetized with 2% Isoflurane and 2 ml 100mM $MnCl_2$ dissolved in saline was delivered through the tail vein (n=6). Control rats did not receive any $MnCl_2$ (n=3). Animal preparation for MR: Rats were anesthetized with 2% Isoflurane and were placed supine (in order to suppress motion) in a secured MR head and body cradle. A 2.0 cm surface coil was placed under the rats thoraco-lumbar vertebrae. MRI: All experiments were carried out in a 7T, 21 cm horizontal Bruker MR imaging system (Bruker, Billerica, MA) equipped with a 72 mm transmitter coil. After acquiring anatomical landmarks in all three planes, 12 coronal slices of 1 mm each spaced 0.2 mm apart were chosen. T_1 weighted spin echo images were acquired using the following parameters: RARE sequence with 2 echoes; TR, 1200 msec; TE, 14 msec; FOV, 2.2 X 2.2 cm with a 320 X 320 matrix. Three saturation slices around the spinal cord area were chosen to allow a reduced FOV around the cord without alias. Fat suppression and spatial saturation modules were used in all experiments. Analysis was performed on Bruker ParaVision 3.0.2 software.

Results and Discussion:

Figure 1 demonstrates T_1 weighted images from control (Fig 1A) and Mn^{2+} infused rat (Fig 1B). Images are from L4 lumbar vertebrae level obtained with 69 microns in-plane resolution. Arrows indicate bright areas that correspond to chord layers. MRI from half the chord from another Mn^{2+} infused rat is shown in Fig. 1C with he corresponding coronal anatomical section (4). Interestingly, the superficial layers which consist mostly of the terminations of primary afferent nociceptive fibers and neurons of lamina I and substantia gelationsa can be visualize in the control rat but are much more enhanced in Mn^{2+} administrated rats. In Mn^{2+} administrated rats, additional T_1 enhancement can be seen in ventro-lateral grey matter, which is a region that contains large motorneurons. Signal enhancement in different spinal cord regions was calculated and normalized according to the nearby muscle. Signal enhancement within grey matter layers and proximate grey matter regions that contain small diameter interneuron populations in Mn^{2+} injected rats and control are demonstrated in **Figure 2**.

Different spinal cord regions are associated with various neurological disorders such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), nerve damage and chronic pain. However, non-invasive high resolution MRI of spinal cord layers has not been demonstrated so far. We show that MEMRI is successful in distinguishing different grey matter areas within the spinal cord and can be used for longitudinal studies of spinal cord physiology *in vivo*.

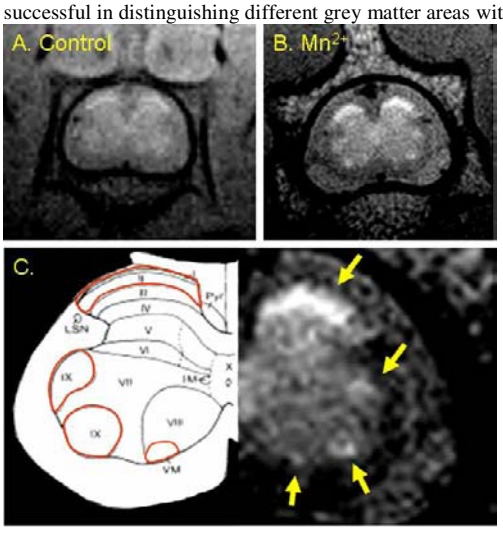

Figure 1. T_1 weighted images of coronal L4 lumbar spinal cord level in control (A) and Mn^{2+} administrated (B) rats acquired 48 hours following Mn^{2+} i.v. administration. (C) shows half of the chord from a Mn^{2+} administered rat with the corresponding anatomical section.

References:

1. Bilgen et al, *MRM*, 2005
2. Pautler & Koretsky, *Neuroimage*, 2002
3. Lee et al, *MRM*, 2005
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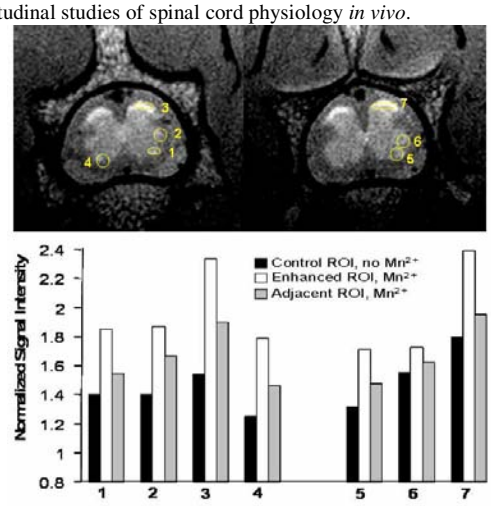

Figure 2. T_1 weighted images of coronal L4 and L5 lumber spinal cord level in Mn^{2+} administrated rat and the normalized (compared to the muscle) signal enhancement in different grey matter regions.