Manganese Enhanced MRI Assay of Spinal Cord Functional Connectivity

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Introduction: Experimental spinal cord injuries in rodents are used to evaluate the potential of cellular grafts for improving cord function (1). Several methods have been developed to trace the neuro-anatomic connections with different neuronal tracers. However, these methods require elaborate tissue analysis based on histology and therefore preclude longitudinal studies in the same animal. Manganese (Mn^{++}) induces strong contrast enhancement in T_1-weighted MRI. Mn^{++} is a divalent ion with chemical properties resembling calcium (Ca^{++}). It is actively transported into neurons via voltage-gated Ca^{++} channels (2). Once inside an axon, Mn^{++} is transported in both anterograde and retrograde directions. Trans-synaptic propagation has also been observed. In previous studies animals treated with the intracerebroventricular and cisterna magna $MnCl_2$ injections resulted in Mn^{++} uptake into the spinal cord (3, 4). Here, introduce Mn^{++} directly into the spinal cord (SC) just rostral to a hemisectioned cord. The aim of this study is to develop a method enabling more objective follow-up of SC injury therapies over time.

Materials and Methods: Adult female mice (C57BL/6, n =3) were anesthetized with isoflurane and placed in a stereotaxic frame. A midline incision was made from thoracic vertebrae 1 to 3. Under a surgical microscope, the dura mater of thoracic vertebrae 2 was opened and the lamina of vertebral levels exposed. A 1mm burr hole was drilled into the right lamina using a 1mm diameter trephine drill bit. 2 µL of 200 mM $MnCl_2$ was loaded in a pulled glass micropipette mounted on a 10 µl Hamilton syringe fixed to a microinjector. The solution was delivered slowly over a period of three minutes through the burr hole. To prevent backflow, the pipette was left in place for another 5 minutes prior to withdrawal. After the injection, mice were kept warm until complete recovery from anesthesia and then re-anesthetized with isoflurane for MRI. MR images were acquired at 11.7T (Bruker BioSpin) using a 20mm RF birdcage coil at 30 minutes, 8, 24, 48 and 72 hrs post injection with a T_1 weighted 3D FLASH sequence (TR/TE, 50/3.4ms; flip-angle 30°; FOV, 220x128x100 mm, Voxel 100 μm^3).

Results and Discussion: In this preliminary study we show the practical implementation of MEMRM aimed at tracing the SC of live mice. Figure 1 shows time-lapse images reconstructed from 3D data over a thickness of 400 µm (4 sagittal slices). Mn^{++} enhanced intensity spread from the injection site to distal locations in the SC over time. At 30 min, Mn^{++}-induced intensity changes are obvious near the injection site. The hypointensity in the injection site results from the T2* effect caused by the local high concentration of Mn^{++}. At a later time points, 8, 24, 48 and 72 hr, Mn^{++} induced intensity has progressed further along the SC. By 72 hr, the cerebral peduncle and pyramidal tract of the brain display increased intensity.

Conclusion: The MEMRI protocol presented here is useful for rapid assessment of SC integrity and can be applied for the evaluation of experimental spinal cord injury treatments to assay axonal outgrowth and functional connectivity.

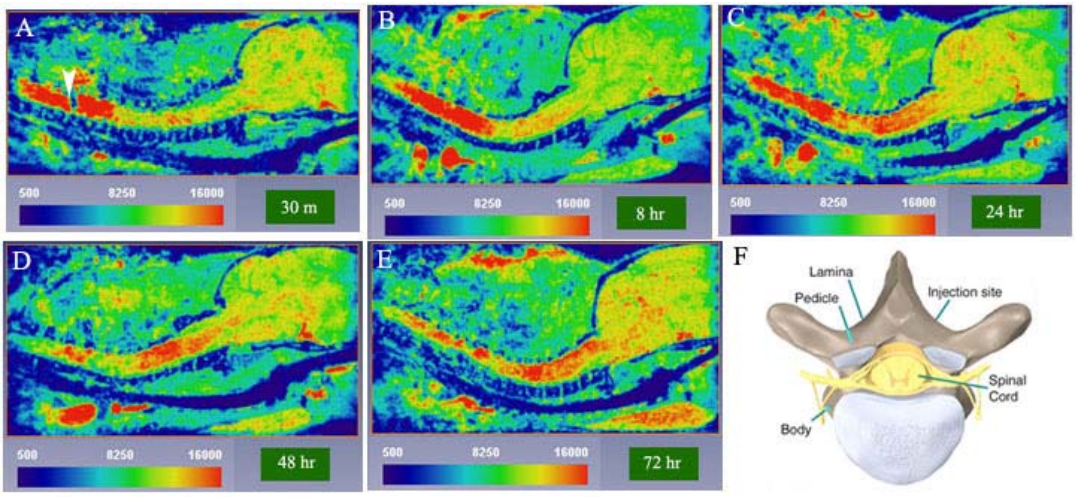

Fig.1.Time-lapse MRI after direct SC injection of Mn^{++} (A-E); at 30 min, intensity enhancement is seen near the injection site (arrowhead). At a later time points, 8, 24, 48 and 72 hr, detectible increases in MR intensity occur gradually over time along the SC. F, spinal cord anatomy.

Ref: [1] Experimental Neurology 2008; 213; [2].NMR Biomed 2004; 17(8); [3] J Neuroscience Methods 2006;156; [4] MRM 2006; 55.