Infusion-Based Manganese-Enhanced MRI: New Imaging Technique to Visualize the Mouse Brain

S. I. Mok[1], J. Munasinghe[2], A. C. Silva[2], and W. S. Young[1]
[1]National Institute of Mental Health, Bethesda, Maryland, United States, [2]National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, United States

Introduction: Manganese-enhanced Magnetic Resonance Imaging (MEMRI) is a technique that employs the divalent ion of the paramagnetic metal manganese (Mn^{2+}) as an effective contrast agent to visualize, *in vivo,* the mammalian brain. As total achievable contrast is directly proportional to the net amount of Mn^{2+} accumulated in the brain, there has been great interest in optimizing administration protocols to increase the effective delivery of Mn^{2+} to the brain while avoiding the toxic effects of overexposure [1]. Recent work has proposed the use of an osmotic pump to achieve continuous slow release of Mn^{2+} in rats [2]. In this study, we employ this method of systemic Mn^{2+} delivery in the mouse brain and examine the effects of different rates of infusion on signal contrast.

Sample: Three cohorts of *C57BL/6J* adult (8 weeks of age) male mice (N=8 per cohort).

Methods: We performed slow systemic infusion of manganese chloride ($MnCl_2$) into the mouse brain via mini-osmotic pumps implanted subcutaneously. Each cohort was assigned one of three $MnCl_2{\cdot}4H_2O$ infusion periods: 3-day (1 uL/hr), 7-day (0.5 uL/hr), or 14-day (0.25 uL/hr). Each treatment provided a cumulative total dose of 180 mg/kg of $MnCl_2{\cdot}4H_2O$ (~3.96-5.4 mg/mouse). All animals were individually housed and provided food and water *ad libitum*. We collected images at two timepoints: pre-infusion and post-infusion. Whole-brain 3D images (echo time=3.5 ms, repetition time=30 ms, isotropic resolution=100 um) were acquired using T_1-weighted gradient echo scans via a 7 Tesla 21 cm horizontal scanner (Bruker Avance, Billerica, MA). All animals were secured in a stereotaxic holder and mounted in a 72 mm volume (transmit) / 25 mm surface (receive) radio frequency coil ensemble.

Results: We observed clear evidence of Mn^{2+} transport at all infusion rates into normally behaving mouse olfactory bulbs, cortex, striatum, cortex, hippocampus, amygdala, hypothalamus, thalamus, and cerebellum (Figure 1). Non-parametric tests conducted on data from ROI analysis of the bilateral amygdala found normalized mean signal in pre-infusion images to be significantly different from that of post-infusion images at the 0.1% confidence level (P<0.001). In addition, mean signal from each of the infusion treatments indicate a trend of increasing signal with higher rates of $MnCl_2{\cdot}4H_2O$ infusion (equal cumulative dose) (Figure 3).

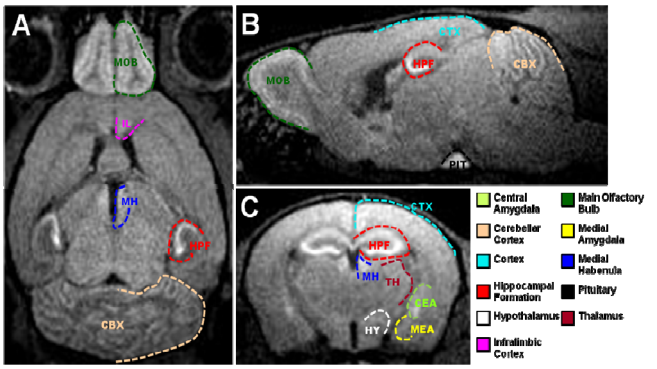

Figure 1. T_1-weighted 3D whole-brain images of a C57BL/6J adult male mouse post-$MnCl_2{\cdot}H_2O$ infusion. (A) horizontal, (B) sagittal, and (C) coronal views with regions of Mn^{2+} accumulation delineated.

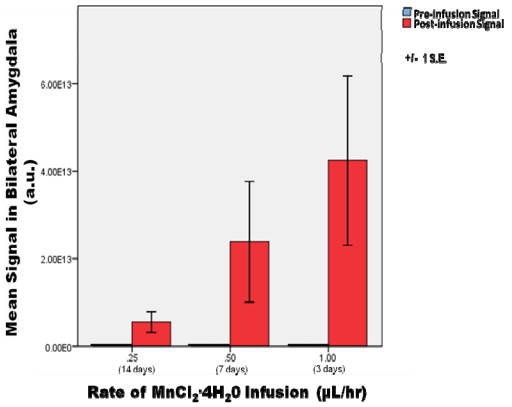

Figure 2. Graph comparing mean signal from the bilateral amygdala (normalized to fiduciary marker) in 14-day, 7-day, and 3-day $MnCl_2{\cdot}4H_2O$ treatments (pre-infusion and post-infusion). Pre-infusion and post-infusion mean signal were significantly different at the 0.1% confidence level (P<0.001). Error bars indicate +/- 1 standard error, N=8.

Discussion: This work demonstrates the successful application of slow systemic infusion to deliver Mn^{2+} to the mouse brain. Moreover, the observation of greater signal intensity with higher rates of $MnCl_2{\cdot}4H_2O$ infusion parallels that of previous studies examining the effects of increasing Mn^{2+}fractionated-dose injections [1]. Importantly, the continuous yet slow release of Mn^{2+} via osmotic pump did not induce any observable toxic effects on animal physiology or behavior (in agreement with previous work examining Mn^{2+} infusion in rats [2]). In this current study, however, we achieved a significantly higher dose of Mn^{2+} (180 mg/kg), which may prove invaluable for future work employing MEMRI to examine behavioral manipulations on functional activity in the mouse brain.

References: [1] Bock et al., NMR Biomedicine. 2008 Jun; 21 (5) 473-8. [2] Eschenko et al., Magnetic Resonance Imaging, 2010 Oct; 28 (8): 1165-74.

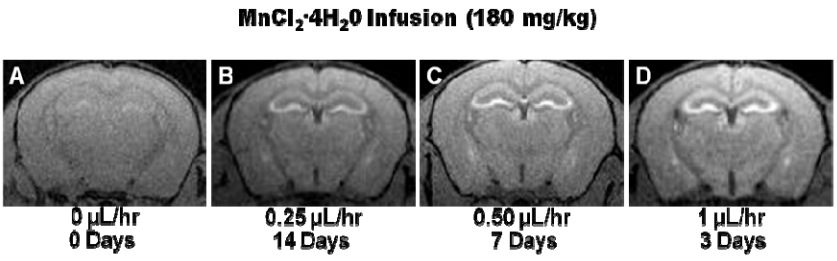

Figure 3. Demonstration of MR signal enhancement with increasing rates of $MnCl_2{\cdot}4H_2O$ systemic infusion. Cumulative dose in all treatments was 180 mg/kg (or approximately 3.96-5.40 mg per adult male mouse). T1-weighted coronal slice images of (A) pre-contrast and post-contrast (B) 14-day, (C) 7-day, and (D) 3-day infusion treatments.