Deconvolving the intra- and extracellular water components in the rat brain using manganese-enhanced MRI (MEMRI)

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Introduction: Diffusion-weighted nuclear magnetic resonance (NMR) techniques have established that the apparent diffusion coefficient (ADC) of cerebral tissue water decreases during ischemia [1-3]. However, it remains unclear whether the ADC change occurs due to changes in the intracellular (IC) space, extracellular (EC) space, or both. Past studies have measured compartment-specific diffusion coefficients using gadolinium as an EC MR contrast agent to distinguish between the IC and EC water signals by reducing the longitudinal (T_1) relaxation time of the EC space [4-5]. In this study, we investigated an alternative approach by using manganese (Mn^{2+}) as an IC contrast agent, which acts as a calcium analogue in rat brain tissue. Mn^{2+} was administered via subcutaneous (SC) injection since a higher Mn^{2+} dose can be delivered compared to other modes of delivery [6]. Mn^{2+} uptake by cells will cause shortening of the T_1 of IC water allowing differentiation between the MR signals arising from water in the respective compartments. This method can provide greater insight about water exchange since the IC space contains majority of the water volume fraction (~80%).

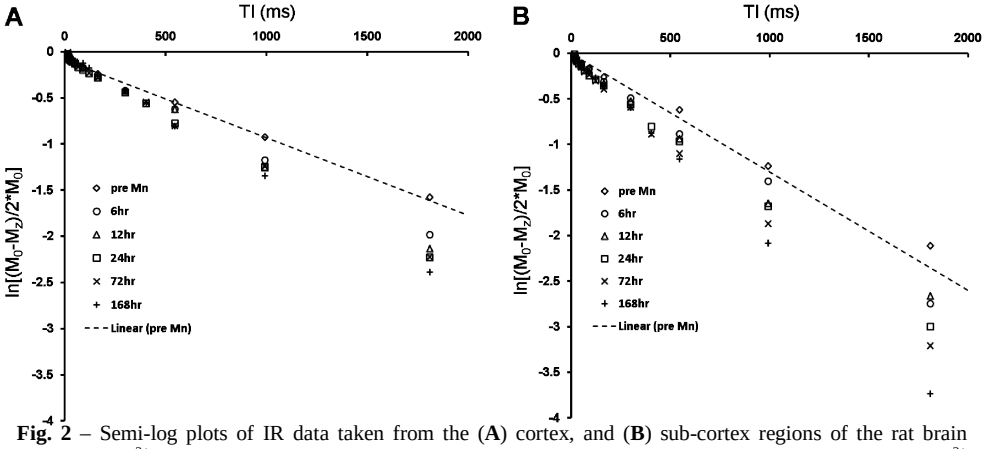

Fig. 2 – Semi-log plots of IR data taken from the (**A**) cortex, and (**B**) sub-cortex regions of the rat brain following Mn^{2+} administration (300mg/kg). Each graph shows IR data plots at different time points after Mn^{2+} injection. The dotted line shows a monoexponential fit through the IR data set prior to Mn^{2+} administration.

$$M_z(t) = M_0(1 - b \cdot e^{\frac{-t}{T_1}}) \quad \text{[Eq. 1]}$$

$$M_z'(t) = M_{0a}(1 - b \cdot e^{\frac{-t}{T_{1a}}}) + M_{0b}(1 - b \cdot e^{\frac{-t}{T_{1b}}}) \quad \text{[Eq. 2]}$$

		Time after Mn^{2+} injection (hrs)				
		6	12	24	72	168
Cortex	M_{0a}	0.95 ± 0.004 (n=7)	0.94 ± 0.004 (n=5)	0.93 ± 0.002 (n=7)	0.95 ± 0.002 (n=7)	0.96 ± 0.003 (n=7)
	T_{1a}	890 ± 11 (n=7)	904 ± 22 (n=5)	850 ± 10 (n=7)	765 ± 9 (n=7)	745 ± 5 (n=7)
	T_{1b}	27 ± 0.1 (n=2)	28 ± 10 (n=2)	33 ± 4 (n=3)	35 ± 3 (n=2)	33 ± 9 (n=2)
Sub-cortex	M_{0a}	0.93 ± 0.004 (n=7)	0.92 ± 0.007 (n=5)	0.94 ± 0.004 (n=7)	0.91 ± 0.006 (n=7)	0.94 ± 0.006 (n=7)
	T_{1a}	747 ± 8 (n=7)	702 ± 23 (n=5)	621 ± 11 (n=7)	559 ± 8 (n=7)	531 ± 4 (n=7)
	T_{1b}	21 (n=1)	31 (n=1)	29 ± 5 (n=4)	47 ± 12 (n=2)	37 ± 9 (n=2)

Table 1 – Biexponential fit results (using Eq. 2) from Fig. 2 plots for M_{0a}, T_{1a} and T_{1b} (±SEM) in the cortex and sub-cortex regions of the rat brain are shown as a function of time after Mn^{2+} administration at 300 mg/kg. Since the IR data was normalized, M_{0b} can be calculated as: $M_{0b}=1-M_{0a}$. The parameter '*b*' in Eq. 2 was ~2 for all fits.

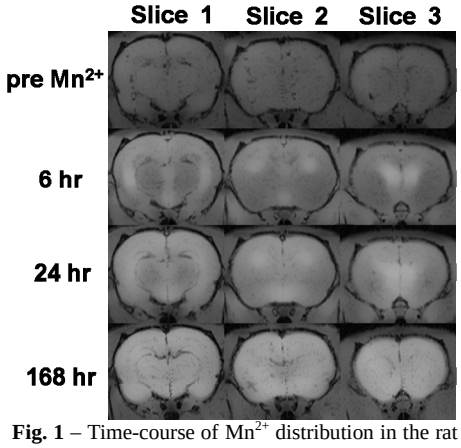

Fig. 1 – Time-course of Mn^{2+} distribution in the rat brain. T_1-weighted axial MR images are shown as a function of time after SC injection of $MnCl_2$ (100mM, dose=300mg/kg).

Methods: Experiments were carried out using 13 male Sprague Dawley rats weighing 200-450 g. $MnCl_2$ was administered using dorsal SC injection at three different doses: 75 (n=3), 150 (n=3), and 300 (n=7) mg/kg. All MR imaging was performed at 2.0T. Multi-slice T_1-weighted (T_1-WT) MR images (TR/TE = 700/15 ms) were acquired pre-injection and 6, 12, 24, 72, and 168 hrs following Mn^{2+} injection. T_1 relaxation times were measured using an inversion recovery (IR) sequence (TR/TE = 10,000/4.8 ms, 16 inversion time (TI) points ranging from 15 ms to 3300 ms) acquired at the same time points as the T_1-WT images. Different brain regions of interests (ROIs) were selected (cortex and sub-cortex) from the MRI slices. A mean ROI value from each TI point was calculated and the IR data set was normalized for monoexponential (Eq.1) and biexponential (Eq. 2) analysis using a combination of curve-stripping and non-linear least squares fitting in MATLAB. χ^2 statistics using an *F* test was used to determine the best fit model.

Results and Discussion: T_1-WT signal enhancement was apparent in the rat brain at 6 hrs which expanded from the ventricles to the sub-cortex and cortex regions by 24 hrs and persisted up to 168 hrs (Fig. 1). At $MnCl_2$ doses of 75 mg/kg and 150 mg/kg, the IR data displayed a monoexponential behavior (data not shown); however, at the highest administered Mn^{2+} dose of 300 mg/kg, the IR data sets seem to exhibit a biexponential behavior after Mn^{2+} administration (Fig. 2). Table 1 lists the average fit values for M_{0a}, T_{1a} and T_{1b} from only the animals that showed a significant fit result. ANOVA test for mixed models showed a significant effect of time point after Mn^{2+} injection ($P < 0.0001$) on the reduction of the long T_1 relaxation times (T_{1a}) in the cortex and sub-cortex regions. The sub-cortex T_{1a} was also significantly less than that of the cortex at all time points indicating greater Mn^{2+} uptake in the sub-cortex region. The short T_1 relaxation times (T_{1b}) and water volume fractions (M_{0a}) did not show a significant difference between time points or ROIs. T_{1a} being associated with the larger volume fraction, M_{0a} (~0.95), and T_{1b} being associated with the smaller volume fraction, M_{0b} (~0.05), is contrary to the hypothesis that Mn^{2+} enters the IC compartment. Several valid reasons could account for this reverse association: 1.) Mn^{2+} could be getting sequestered in the IC organelles, thereby not affecting IC water T_1; 2.) IC T_2 signal from Mn^{2+} entry might be getting attenuated making it difficult to decipher IC T_1; 3.) greater concentrations of Mn^{2+} might be required to further shift the water exchange system from the fast to the slow exchange regime (SXR).

Conclusion: Mn^{2+} injected at a high SC dose appears to give a local Mn^{2+} concentration in the brain that might be sufficient to achieve SXR for water exchange in the rat brain. Biexponential T_1 behavior was observed in several ROIs and time points only at the highest administered Mn^{2+} dose (300 mg/kg) possibly indicating a distinction between the IC and EC spaces; however, the corresponding signal fractions did not agree with the known water volume fractions of cerebral tissue. Despite that, this approach, when combined with diffusion measurements, could allow separate measurements of the corresponding component ADCs under both normal and pathological conditions.

References: [1] Wesbey *et al.* (1984). Invest Radiol **19**: 491-498; [2] Le Bihan *et al.* (1986). Radiol **161**: 401-407; [3] Moseley *et al.* (1990). Magn Reson Med **14**: 330-346; [4] Silva *et al.* (2002). Magn Reson Med **48**: 826-837; [5] Silva *et al.* (2002). J Magn Reson **156**: 52-63; [6] Shazeeb *et al.* (2012) Magn Reson Med doi: 10.1002/mrm.24184.