

Variations in Relaxivity of Manganese between Regions in Rat Brain

N. Zhang^{1,2}, V. A. Fitsanakis³, M. Aschner^{3,4}, M. J. Avison^{1,5}, J. C. Gore^{1,2}

¹Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, Tennessee, United States, ²Physics Department, Vanderbilt University, Nashville, Tennessee, United States, ³Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, United States, ⁴Department of Pharmacology and the Kennedy Center, Vanderbilt University Medical Center, Nashville, Tennessee, United States, ⁵Neurology Department, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Introduction: Manganese (Mn) is an essential metal that is neurotoxic at high levels but which has been used with MRI *in vivo* to track neuronal architecture [1]. As part of a toxicological study that aims to determine the pattern of Mn distribution in rat brain following weekly injections of Mn, we measured the effective relaxivity of Mn *in vivo* in different regions of the brain. The variations found suggest Mn takes different chemical or physical forms in different parts of the brain, which may be relevant for studies that assume the relaxivity is constant.

Theory: Mn²⁺ is a strongly paramagnetic ion capable of shortening both T1- and T2-of water protons in solutions [2, 3]. A linear relationship usually exists between the concentration of Mn²⁺ and the relaxation rates R1 and R2: $R_{1,2}([Mn^{2+}]) = R_{1,2}(0) + k_{1,2}[Mn^{2+}]$ in free solutions, where $k_{1,2}$ is the relaxivity [3]. However, the relaxivity is not an intrinsic property of the metal ion alone. $k_{1,2}$ may change with the specific molecular form and the environment in which the metal resides. For example, it may increase if the rotational correlation time increases as a result of binding to large molecules, while it may decrease if the metal's electron structure is altered or if the water's access to the metal is restricted, as occurs in many molecular configurations [2,3]. Quantification of the ratio k_2/k_1 may also provide insights into the chemical form of the metal [2]. Such effects imply that the relationship between relaxation rate and metal concentration may vary between tissues and complicate the potential use of relaxometry for estimating metal levels.

Methods: Treated rats received weekly intravenous injections of isotonic Mn²⁺ solution equivalent to 3 mg Mn/kg body weight for a total of 14 weeks. These correspond to doses of Mn comparable to those acquired by humans exhibiting symptoms of Mn intoxication. Both control and treated groups were scanned every other week. T1 and T2 values were measured from regions of interest (ROIs) in rat brain images acquired using a 4.7 T Varian imaging system. FSE and FLASH sequences were used to calculate T2 and T1 respectively. At the end of the study, the animals were euthanized, and their brains were removed and dissected to correspond with the specific ROIs. Mn concentrations in the brain samples were measured with graphite furnace atomic absorption spectroscopy (AAS). Multiple MnCl₂ phantoms with similar T1 and T2 values as the rat brain tissues were also made (pH =7). Their T1 and T2 values were measured at 37 °C using an inversion recovery method and by changing TE in a spin echo sequence respectively.

Table 1: Relaxivities ($s^{-1}mM^{-1}$) for MnCl₂ phantoms and Mn in Different Brain Regions*

	Phantom	Hippocampus	Striatum	Cerebellum	Brainstem	Midbrain	Cortex
k_1	8.3899	0.49592	0.6608	2.063	3.1944	0	0
k_2	145.82	0	3.192	8.32	25.8896	0	0
k_2/k_1	17.38	0	4.83	4.03	8.10	N/A	N/A

*Notes: For better comparison, the relaxivities of brain tissues are changed to units of ($s^{-1}mM^{-1}$) by assuming 80% water content in brain tissues.

Results: Student's t-tests were used to compare the differences in Mn levels in each region between the control and the treated groups. Significant increases in Mn accumulation were observed in the cerebellum ($p < 0.0001$), brain stem ($p = 0.0039$), and hippocampus ($p = 0.0018$); Mn increases in striatum approached significance ($p = 0.0858$), whereas midbrain ($p=0.6025$) and cortical metal levels ($p=0.908$) did not change (Figure 1). The relaxation rates in most tissues increased linearly with increasing Mn levels (Figure 2). However, the slopes (which may indicate the effective relaxivities) were very different in different regions and are all much smaller than the relaxivities of Mn in a simple solution. In addition, the ratio of R2 relaxivity to R1 relaxivity also varied between regions and was less than the ratio of the solution (Table 1.).

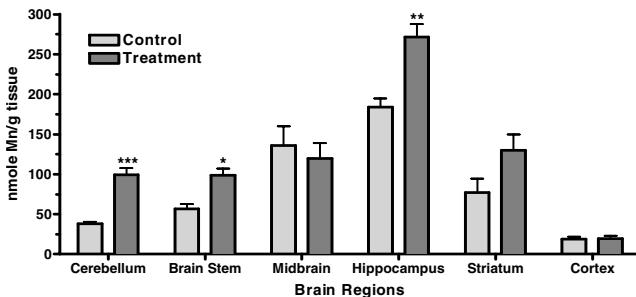


Figure 1. Brain Manganese Accumulation (AAS)

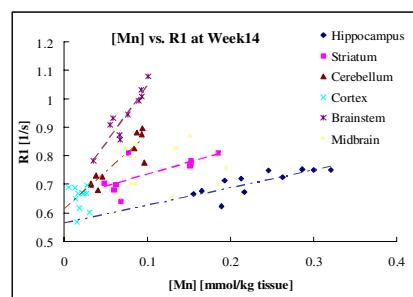


Figure 2. [Mn] vs. R1 at Week 14

Discussion: The increases in relaxation rates are likely mainly due to the deposition of manganese in the brain, though other contributions may be present e.g. from iron. If the increases are caused by the manganese then the relaxivities of Mn in brain areas are much less than those of the free ion and vary with region. The observation that Mn relaxivity varies among regions suggests the metal exists in different forms, or is physically confined to different degrees in different areas. The presence of difference substrates for binding and the effects of competition from other metals, as well as the variation in microviscosity may all influence the relaxivity [2, 3]. Similarly, the k_2/k_1 ratio suggests there are differences in chemical form and binding effects. In hippocampus, we measured no significant change of R2, while the R1 relaxivity was also small, although the metal levels there were relatively high. This implies that the relaxation effect of the Mn as a paramagnetic ion is largely decreased there, as would occur if the Mn²⁺ metal is converted to a nonparamagnetic state or if the water access to the ion is restricted. Similar mechanisms may also exist in midbrain. These regional variations may be important for the interpretation of MR methods in use for tracking neural connectivity and for the use of MRI for monitoring metal deposition in tissues.

References:

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