Reduced intracellular mobility underlies manganese relaxivity in mouse brain *in vivo*: MRI at 2.35 and 9.4 T

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Target Audience Anyone who is interested in manganese-enhanced MRI [1], MRI contrast agents, or MRI of manganese poisoning.

Purpose (i) To perform T_1-weighted MRI analyses at two different field strengths and to explore if such measurements provide additional insights into the cellular localization and chemical environment of Mn^{2+} in mouse brain *in vivo*, (ii) to estimate the enhancement factor ε* and relaxivity r_1 for Mn^{2+} in striatum *in vivo*, and (iii) to assess the access of Mn^{2+} and Gd-DTPA to extra- and intracellular compartments after disruption of cellular membranes.

Methods *T_1 measurements.* (i) 9 mice received a single intraventricular injection of Gd-DTPA (5 µL, 100 mM). 3 hours later, the T_1 of 5 of these animals was measured at 2.35 T, while the other 4 were measured at 9.4 T. In addition, these 4 mice underwent high-resolution T_1-weighted MRI (see below) *in vivo* as well as *post mortem.* (ii) Another 6 mice received a single s.c. injection of $MnCl_2$ (0.5 mmol/kg b.w.). One (n=4), 3 (n=4), and/or 7 (n=3) days later, T_1 was measured at 9.4 T followed by 2.35 T. (iii) Another 4 mice received multiple s.c. injections of $MnCl_2$ (0.25 mmol/kg b.w. on days 1, 4, and 7). On days 8, 10, and 14, T_1 was measured at 9.4 T and 2.35 T. T_1 was determined with multiple TR spin-echo MRI (in-plane resolution 117 µm, slice thickness 234 µm). Values for cerebral (prelimbic) cortex, striatum, thalamus, and cerebellar cortex were obtained with TE 16 ms (2.35 T) and 10 ms (9.4 T) and 7 TR (0.2—10 s). Relaxivities r_1 are defined as $\Delta R_1\ \Delta[\text{concentration}]^{-1}$ with ΔR_1 the relaxation rate increase and Δ[concentration] the concentration increase of a contrast agent at a certain point of time after injection. Because it may be assumed that Δ[concentration] are identical for the same animals undergoing MRI at the same time after receiving the same dose of the agent through the same route, the corresponding **relaxivity ratios RR** at the two field strengths may simply be calculated by $^{100}\mathbf{r_1}/^{400}\mathbf{r_1} = {}^{100}\Delta\mathbf{R_1}/^{400}\Delta\mathbf{R_1}$. (iv) Aqueous relaxivities r_1 (in $s^{-1}\ mM^{-1}$) were determined by measuring T_1 for 5 different concentrations of Mn^{2+} (0.05—0.5 mM) and Gd-DTPA (0.1—1.0 mM) at both 20 ºC and 37 ºC.

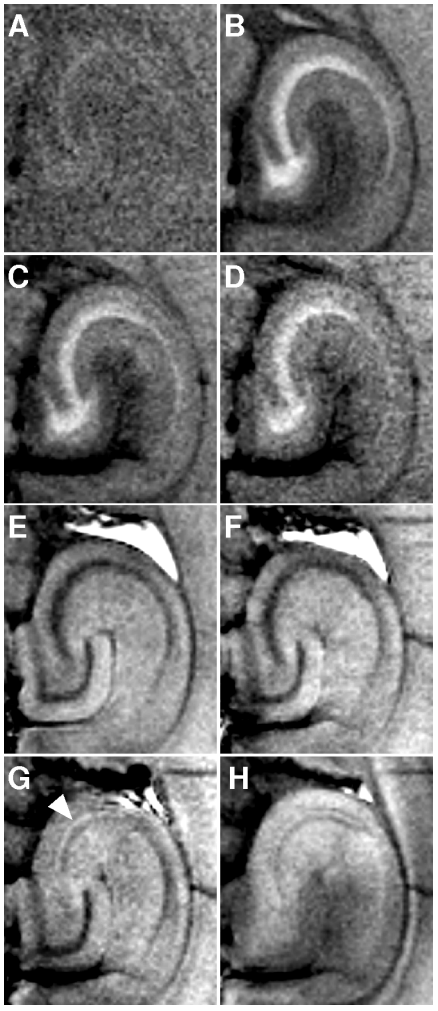
Enhancement factors for Mn^{2+} in the striatum. Additional 4 mice received the multiple s.c. injections and underwent the T1 measurements. $^{100}r_1$ and $^{400}r_1$ were obtained from $^{100}\Delta R_1$ and $^{400}\Delta R_1$ on the assumption of 4.0 µg/g tissue = 0.073 mmol/kg tissue as Δ[concentration] of Mn^{2+} with other factors remaining constant [2]. $^{100}\varepsilon^*$ and $^{400}\varepsilon^*$ were obtained as ratios of $^{100}r_1$(in vivo)/$^{100}r_1$(aqueous) and $^{400}r_1$(in vivo)/$^{400}r_1$(aqueous) with aqueous values at a temperature of 37ºC.

High-resolution MRI. Additional 4 mice underwent T_1-weighted MRI at 9.4 T. RF-spoiled 3D FLASH (TR/TE = 23/7.6 ms, α 25°, resolution 30 × 30 × 300 μm^3) were performed 3 days after a single s.c. injection of $MnCl_2$ (0.5 mmol/kg b.w.) *in vivo* as well as *post mortem.*

Results and Discussion **Table** below summarizes the T_1 relaxation data (T_1 in seconds, mean ± SD. $^{\#}\mathbf{RR} = {}^{100}\mathbf{r_1}/^{400}\mathbf{r_1} = {}^{100}\Delta\mathbf{R_1}/^{400}\Delta\mathbf{R_1}$) as observed for mouse brain *in vivo* before and after injections of Mn^{2+} or Gd-DTPA at 2.35 T and 9.4 T. Both contrast agents shorten the T_1 in all brain tissues and at both field strengths. However, while $^{100}\Delta R_1$ and $^{400}\Delta R_1$ for Gd-DTPA are very similar at 2.35 T and 9.4 T, the values for manganese are up to 4-fold smaller ($P<0.05$) at the 4-fold higher field. **In aqueous solution, the relaxivities $^{100}\mathbf{r_1}$ and $^{400}\mathbf{r_1}$** for Gd-DTPA are 4.8 and 4.4 at 20ºC as well as 3.8 and 3.7 at 37ºC, while for Mn^{2+} the results are 5.4 and 5.0 at 20ºC as well as 3.9 and 3.9 at 37ºC, respectively. Thus, **all relaxivity ratios $^{100}\mathbf{r_1}/^{400}\mathbf{r_1}$ range between 1.0 and 1.1 except for Mn^{2+} *in vivo***, where respective values range from 2.4 for the striatum to 4.4 for the cerebellar cortex. In the striatum at one day after 3 injections, the relaxivities $^{100}r_1$ and $^{400}r_1$ are 9.2 and 4.5 kg $mmol^{-1}\ s^{-1}$ yielding a relaxivity ratio $^{100}r_1/^{400}r_1$ of 2.1. Thus, $^{100}\varepsilon^*$ and $^{400}\varepsilon^*$ are 2.2 and 1.1, respectively, because aqueous Mn^{2+} values of $^{100}r_1$ and $^{400}r_1$ of 3.94 and 3.86 $mM^{-1}\ s^{-1}$ at 37ºC correspond to 4.1 and 4.0 kg $mmol^{-1}\ s^{-1}$, respectively. These results suggest Mn^{2+} ions *in vivo* are in a viscous fluid and/or bound to macromolecules, because a reduced mobility increases the longitudinal relaxation rate by eliminating the contribution of the rotational correlation time in the frequency range 10 – 100 MHz [3].

High-field data show that Mn^{2+} is most concentrated in brain one day after repeated injections. $^{400}\Delta R_1$ is considered to be a more reliable measure of Mn^{2+} concentration due to a lower relaxivity enhancement than $^{100}\Delta R_1$. For a single injection, Mn^{2+} concentration is highest 3 days after injection. One day after the single injection $^{100}r_1/^{400}r_1 = 2.8$ for brain Mn^{2+} is significantly ($p < 0.005$) higher than $^{100}r_1/^{400}r_1 = 1.7$ after 3 injections despite a lower brain concentration indicated by a significantly lower $^{400}\Delta R_1$. At later stages $^{100}r_1/^{400}r_1 \leq 2.5$, while the assumed brain concentrations (i.e., $^{400}\Delta R_1$) remain high. These discrepancies indicate that the accumulation of Mn^{2+} in the brain is not a single process which proportionally reduces the mobility. Instead, it suggests that effective immobilization during early exposure to excessive Mn^{2+} is accompanied by a separate Mn^{2+} "storage" with less immobilization in order to minimize the disturbance of cellular function by isolating Mn^{2+} from physiologically relevant macromolecules. While acute manganese poisoning in human may be related to the immediate dysfunction of macromolecules, neurodegenerative processes associated with chronic manganese poisoning may involve an isolation or confinement mechanism.

The Mn^{2+}-induced contrast for cell layers *in vivo* persists *post mortem* substantially longer than that induced by Gd-DTPA. **Figure** (right) shows T_1-weighted MRI (9.4 T) of the hippocampus in horizontal sections: (A) before and (B) 3 days after Mn^{2+} injection *in vivo*, (C) 90–102 min *post mortem*, (D) 120–132 min *post mortem.* (E) 220–232 min after Gd-DTPA injection *in vivo* as well as (F) 0–12 min, (G) 30–42 min, and (H) 90–102 min *post mortem.* Mn^{2+} accumulates predominantly in the pyramidal cell layers (A, B). After death, Mn^{2+} remains for at least 2 hours (C, D). The Gd-DTPA contrast persists for several hours *in vivo* after injection (E). During the first 12 min after death, Gd-DTPA mostly remains in extracellular spaces (F), but within 42 min Gd-DTPA enters the CA3 pyramidal cells (G; white arrowhead) and within 102 min all cell layers are affected (H). These observations are in line with a binding of Mn^{2+} to intracellular compounds and/or a sequestered intracellular storage, because otherwise smaller Mn^{2+} would rapidly move through the impaired outer plasma membrane according to the concentration gradient.



Conclusion The combined application of low-field and high-field MRI identified a reduced mobility of Mn^{2+} as a mechanism underlying the enhanced relaxivity *in vivo*. Pertinent studies may be exploited for a characterization of novel contrast agents *in vivo* as well as for studying neuropathologic alterations as e.g. seen in manganese poisoning.

References [1] Koretsky AP, Silva AC, 2004. *NMR Biomed* 17:527 [2] Dodd CA et al., 2005. *Int J Toxicol* 24:389 [3] Lauffer RB 1987. *Chem Rev* 87: 901.

Manganese	**2.35 T (n=4)**			**9.4 T (n=4)**			
	T_1 Before	**T_1 After**	**$^{100}\Delta R_1$**	**T_1 Before**	**T_1 After**	**$^{400}\Delta R_1$**	**RR$^{\#}$**
Cerebral C.	1.43±0.02	0.87±0.10	0.46	1.98±0.06	1.46±0.06	0.18	**2.6**
Striatum	1.24±0.10	0.73±0.08	0.56	1.85±0.10	1.27±0.14	0.25	**2.4**
Thalamus	1.22±0.06	0.76±0.06	0.50	1.67±0.05	1.27±0.08	0.19	**2.7**
Cerebel. C.	1.38±0.14	0.79±0.04	0.54	1.86±0.02	1.49±0.08	0.14	**4.4**
Gd-DTPA	**2.35 T (n=5)**			**9.4 T (n=4)**			
	T_1 Before	**T_1 After**	**$^{100}\Delta R_1$**	**T_1 Before**	**T_1 After**	**$^{400}\Delta R_1$**	**RR**
Cerebral C.	1.30±0.10	0.54±0.08	1.2	1.92±0.06	0.65±0.11	1.1	**1.1**
Striatum	1.24±0.08	0.80±0.10	0.48	1.82±0.11	0.98±0.03	0.47	**1.0**
Thalamus	1.19±0.03	0.61±0.09	0.88	1.72±0.08	0.70±0.11	0.87	**1.0**
Cerebel. C.	1.29±0.15	0.52±0.05	1.2	1.77±0.13	0.58±0.04	1.2	**1.0**