Contrast-Enhanced Magnetization Transfer MRI at 9.4 T: Myelin Mapping in the Central Nervous System of Living Mice

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Introduction Myelin has a higher lipid content and a lower water content than the rest of the brain tissue. The protons of respective lipids are highly immobilized. Their resonant frequencies range over tens of kilohertz. Exploiting their wide-ranging resonant frequencies, the magnetization transfer (MT) MRI [1] has proven useful for mapping myelin-rich white matter (WM). In general, the saturation of water protons by MT competes with the T_1 recovery. Therefore, an intraventricular injection of Gd-DTPA with or without a systemic administration of $MnCl_2$ improves the contrast in MT MRI at 2.35 T [2] because these T_1-shortening agents can be preferentially delivered to gray matter (GM). Extending this work, the aims of this study were (i) to examine whether such a contrast enhancement can also be achieved at 9.4 T, (ii) to estimate the extracellular space in WM/GM of the brain *in vivo* in order to gain an insight into the contrast enhancement, and (iii) to obtain contrast-enhanced images of the brain and the spinal cord *in vivo* with high spatial resolution at 9.4 T in order to compare them with conventional *ex vivo* histological images.

Methods A total of eleven mice (NMRI, 8-16 weeks, 30-46g) were used.

(i) Experimental validation at 9.4 T. Four mice received an intraventricular injection of Gd-DTPA solution (5.0 µL, 100 mM, Magnevist® diluted in physiological saline, Schering, Berlin, Germany). MT MRI was performed before and 130 min after the injection. For a reproducible and reliable fixation of the mouse head and the radio frequency (RF) coils in the isocenter of the magnet, the Göttingen animal bed [3] was used. An off-resonance RF irradiation with 2.5 kHz frequency offset, 100 Hz mean amplitude, and 12 ms duration was incorporated into gradient-echo MRI (3D FLASH, TR/TE 23/4.6 ms, α 5°) at an isotropic resolution of 117 µm. MT ratio was obtained from acquisitions with and without the off-resonance irradiation. For evaluation, regions-of-interest were selected in WM (corpus callosum, external capsule, fimbria, ventral hippocampal commissure, cerebellar WM) and in GM (prelimbic cortex, thalamus, hippocampal formation, cerebellar cortex).

(ii) Estimation of the tissue extracellular space. Five mice were used. Before and after the intraventricular Gd-DTPA injections, T_1 of WM (corpus callosum and cerebellar WM), GM (prelimbic cortex, striatum, thalamus, cerebellar cortex), and the cerebrospinal fluid (CSF), were determined at 2.35 T using a spin-echo multiple TR saturation recovery method. To determine the longitudinal relaxivity (r_1) of Gd-DTPA, T_1 of its aqueous solution (55 ml, 37ºC) with different concentrations (0.1, 0.2, 0.3, 0.5, and 1.0 mM) were determined. On the assumption that the equilibration of Gd-DTPA between CSF and the tissue extracellular fluid occurs and that the water of pertinent tissue compartments is in fast exchange, the tissue extracellular space was estimated as ΔR_1 (the increase in the relaxation rate by Gd-DTPA injection) in tissue / ΔR_1 in CSF. The Gd-DTPA concentration was estimated as $\Delta R_1/r_1$, with an approximation of r_1 in the extracellular fluid to r_1 in water.

(iii) High-resolution MT MRI at 9.4 T. Two mice (mouse no. 10 and 11) received a subcutaneous injection of $MnCl_2$ (0.3 mmol/kg body weight) before receiving an intraventricular Gd-DTPA injection (3.0 µL, 100 mM) 3 days later. At 90 min after the Gd-DTPA injection, the MT MRI data (see above) at an isotropic resolution of 60 µm were acquired from the mouse no. 10 / 11 with a quadrature / 4-channel phased-array surface coil with 153 / 102 min measuring time, respectively.

Results and Discussion *(i)* At 9.4 T (similar to 2.35 T [2]), the delineation of WM with the use of MT contrast was considerably improved after the intraventricular injection of Gd-DTPA (n = 4). The injection increased the mean SNR by 25% in WM but 46% in GM, which resulted in a mean CNR improvement between WM and GM by 85%. The mean MT ratio was reduced by 21% (from 0.57 to 0.45) in WM but by 48% (from 0.47 to 0.24) in GM.

(ii) The extracellular spaces in WM and GM were estimated to be 15% and 27%, respectively. With the r_1 determined to be 3.8 $(mM \cdot s)^{-1}$, the Gd-DTPA concentrations in WM, GM, and CSF were estimated to be 0.13, 0.22, and 0.82 mmol/l, respectively. The lower/higher concentration of Gd-DTPA as a result of the smaller/larger extracellular space explains the less/more pronounced shortening of T_1 relaxation times in WM/GM, respectively.

(iii) In MT MRI with a 60 µm isotropic resolution at 9.4 T, the delineation of WM with the use of MT contrast is considerably improved after the combined use of $MnCl_2$ and Gd-DTPA, as shown in **Fig. 1** (left and middle column). Their combined use increased the SNR by 46% in WM but by 67% in GM, which resulted in a CNR improvement between WM and GM by 136%. The underlying T_1 shortening due to the paramagnetic agents reduced the mean MTR by 34% (from 0.56 to 0.37) in WM but by 62% (from 0.49 to 0.18) in GM. Thus, together with the use of the phased-array coil, as shown in **Fig.1** (right column) and **Fig. 2** (middle column), contrast-enhanced MT MRI provided a 60 µm isotropic resolution with sufficient CNR within 102 min. In the thalamus (**Fig. 2**, top and middle rows), minor myelinated fibers, e.g., the fornix, mammillothalamic tract, fasciculus retroflexus, and medial lemniscus (arrows and arrowheads), are now more clearly delineated. Within the cerebellar cortex (**Fig. 2**, bottom rows), the granular layer (white asterisks), which is known to contain myelinated fibers, is delineated from the molecular layer (black asterisks), which contains no myelinated fibers. A considerable improvement of WM/GM contrast was also observed in the cervical spinal cord as shown in **Fig. 3**.

In conclusion, at 9.4 T, administration of Gd-DTPA and $MnCl_2$ improve the delineation of myelinated structures in MT MRI of the central nervous system of mice *in vivo*.

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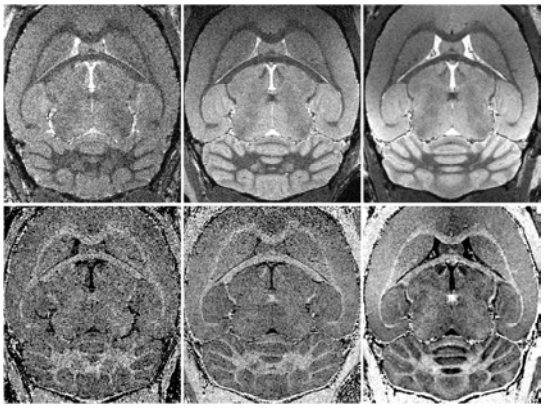

Fig. 1 The effect of the combined use of Gd-DTPA and $MnCl_2$ on (top row) MT MRI and (bottom row) MT ratio maps in horizontal sections: (left column) a 153 min acquisition of the mouse no. 10 with the use of a quadrature coil before and (middle column) after the injections. (Right column) after the injections, a phased-array coil allows for a shorter (102 min) acquisition time with sufficient CNR at 60 µm isotropic resolution (the mouse no. 11).

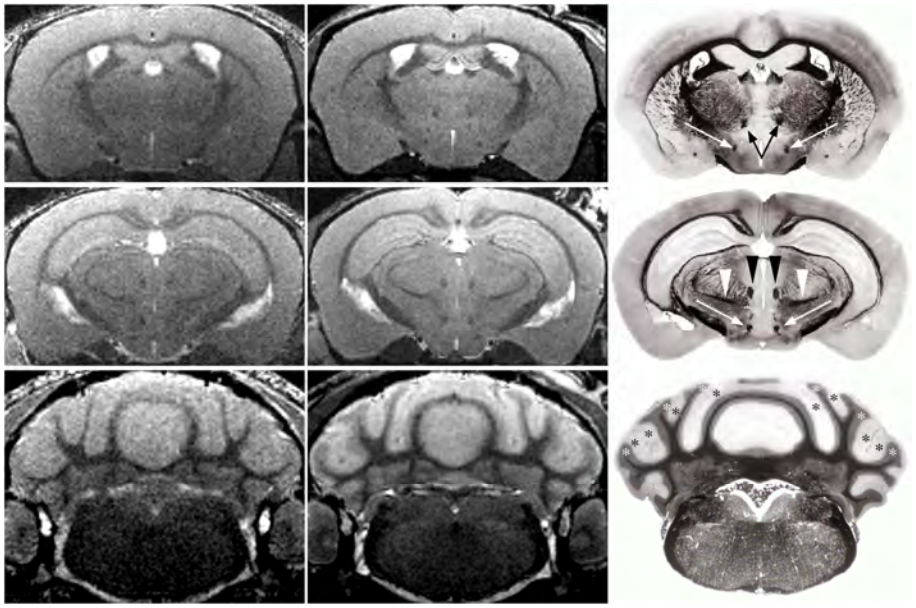

Fig. 2 MT images (60 µm isotropic resolution) of the brain of the mouse no. 11 *in vivo* (left column) before or (middle column) after the injections of $MnCl_2$ and Gd-DTPA, and (right column) corresponding coronal histological sections *ex vivo* stained for myelin [4]. White arrows = fornix, black arrows = mammillothalamic tract, black arrowheads = fasciculus retroflexus, white arrowheads = medial lemniscus, black asterisks = molecular layer, white asterisks = granular layer.

Fig. 3 MT images of the cervical spinal cord of the mouse no. 11 *in vivo* (left) before or (middle) after the use of Gd-DTPA and $MnCl_2$, and (right) a corresponding axial histological section *ex vivo* [5].