Manganese-enhanced MRI in a mouse model of de- and remyelination
Susann Boretius1,2, Tanja Ducic3, Angelika Escher4, Christine Stadelmann4, and Jens Frahm1

1Biomedizinische NMR Forschungs GmbH, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany, 2Klinik für Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein, Kiel, Germany, 3Hasylab, DESY, Hamburg, Germany, 4Institute of Neuropathology, University Medical Center, Georg-August-University, Göttingen, Germany

Introduction: Due to their calcium-like behavior and shortening of T1 manganese ions (Mn2+) are widely used in experimental animal research to visualize brain structures, neuronal activity and connectivity [1]. However, only little is known about Mn2+ accumulation under pathological conditions. Here we took advantage of the well characterized mouse model of cuprizone-induced toxic de- and remyelination [2] and analyzed the Mn2+ accumulation in the brain by in vivo MRI at different time points of cuprizone treatment. Selected tissue samples were analyzed by synchrotron based X-ray fluorescence microscopy.

Methods: Twelve C57BL/6 mice at the age of 8 weeks were fed with 0.25% cuprizone in ground breeder chow ad libitum over 2 weeks (group A, n = 4) and 4 weeks (groups B and C, n = 4 each), respectively. After withdrawal of cuprizone, group C received normal food for additional 10 days. Two additional mice served as controls and were maintained on a normal diet for the duration of the experiment. At the respective end of treatment MRI was performed on isoflurane-anesthetized mice before and 24 h after MnCl2 (40 mg/Kg, i.p.) administration using a 3D spoiled FLASH sequence (TR/TE=17/3.6 ms, flip angle 25°, 100 µm isotropic resolution). In addition, one mouse per group underwent MRI at 2 h and 8 h after Mn2+ injection. All measurements were performed at 9.4 T (Bruker Bio Spin, Germany). After MRI, mice were sacrificed for histology including immunohistochemical staining of amyloid precursor protein (APP), anti-glial fibrillary acidic protein (GFAP), CD3+, and MAC3+ cells.

Results and Discussion: Compared to controls (Fig. 1, control) no alterations of Mn2+ distribution were observed at 2 weeks of cuprizone treatment (Fig. 1, 2w). In contrast, after 4 weeks of treatment, 24 h after Mn2+ administration a distinct signal enhancement was observed mainly at the anterior commissure and the outer layers of the corpus callosum (Fig. 1, 4w, arrows). After 10 days of recovery and remyelination this signal enhancement was decreased (Fig. 1, 4w+10d, arrows). At that time point distinct accumulations of Mn2+ appeared in the striatum as shown in a horizontal section (Fig 2. 4w+10 d, arrows). This pattern of signal enhancement after Mn2+ administration was correlated with microglia activation (Fig. 2, bottom left: MAC3 staining, right: MRI 24 h after Mn2+).

Synchrotron based X-ray fluorescence microscopy revealed a point-shaped, cell-body like accumulation of Mn2+ (Fig. 3 A, arrows, Mn – blue, Fe – red) and a co-localization with Ca2+ (Fig. 3 B, arrows, Mn – blue, Ca – red). The fact that MRI signal enhancement was first observed at about 8 h after Mn2+ injection, indicates an unaffected permeability of the blood brain barrier for Mn2+ (Fig. 4).

Summary: Mn2+-enhanced MRI revealed a distinct Mn2+ accumulation in specific brain regions that refers to activated microglia cells as evidenced by immunohistochemistry. X-ray fluorescence spectroscopy demonstrated a co-localization of manganese with calcium in cell-body like structures that most likely represent microglia cells and no accumulation in neuronal fibers. Although Mn2+ is often used to measure neuronal activity, a possible bias in animal models with inflammation has to be taken into account. The specific accumulation of manganese indicates that manganese-enhanced MRI may significantly contribute to the understanding of immune-mediated diseases in the CNS and potentially also in other tissues.