Contribution of gliosis, neurodegeneration, and axonal spouting to the Manganese Enhancement MRI contrast in the rat hippocampus after systemic Mn administration

R. J. Immonen[1], A. Pitkänen[1], and O. H. Gröhn[1]

[1]Department of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, Kuopio, Finland

Introduction

Mn^{2+}-enhanced magnetic resonance imaging (MEMRI) has the potential to reveal functional, structural and connectional alterations in high spatial resolution [1]. Intracerebral Mn injection to entorhinal cortex was recently used to characterize activity-dependent plasticity in the mossy fiber pathway after status epilepticus in rats [2]. It was also shown that Mn^{2+} enters the hippocampus after systemic administration [3]. In our previous work we hypothesized that systemic Mn injection may provide a robust MEMRI approach for detection of mossy fiber sprouting in epileptogenic rats [4]. Results, indeed, showed alterations in the manganese contrast of hippocampal areas known to have abnormal axonal sprouting: dentate gyrus (DG), CA1 and CA3. However, these same areas also displayed other alterations, such as gliosis and neuronal loss, which may affect the MEMRI contrast. The present study was designed to evaluate the contribution of these features to the MEMRI contrast after systemically injecting Mn into the hippocampus of epileptogenic rats.

Methods

Kainic acid (KA) injections (10 mg/kg, i.p.), which induced status epilepticus, and launched epileptogenesis, was given to 17 Spraque Dawley –rats. Eight saline injected rats served as controls. The systemic manganese injection ($MnCl_2$ in bicine buffer, 45mg/kg, i.p.) was given 24 hours before the imaging. MRI was performed under 1% halothane anaesthesia, 4 days (N=5), 12 days (N=1) and two months (N=17) after KA injection. Immediately after the imaging the animals were transcardially perfused and stained for histology. MRI data were acquired at 4.7T using Varian Inova console and a quadratur half volume RF-coil. T1-wt 3D images were collected using a gradient echo –sequence (TE=2.7 ms, TR=120 ms,) incorporating an adiabatic 70-degree BIR-4 excitation pulse to reduce influence of B1 inhomogeneity. Volume of 25* 25*35 mm^3 was covered with 192*64*256 points. Data for T1-maps were acquired from a single slice using IR-fast spin echo -sequence (TR=4s, echo spacing=13ms, 8 echoes, 128*256pts, FOV=2.0*2.56 cm^2, thk=0.7mm; TI=10, 400, 1000, 1600 ms). In histology the Nissl staining was used to evaluate the neuronal loss and gliosis. The percentual increase / decrease of neurons and glia in TBI animals was evaluated compared to controls. Timm staining was used to quantify mossy fiber sprouting from the granule cell layer of dentate gyrus (scoring from 0-5 according to Cavasos decription [5]).

Results and Conclusions

In following, detailed description of the MEMRI changes and histological findings in hippocampal substructures are given for 6 representative rats.

Control animals showed typical MEMRI enhancement of hippocampal structures 24 h after Mn injection and appeared normal in histology (Fig 1A).

At 4 days after KA injection there was no detectable mossy fiber sprouting in histology. However, significant neuronal loss (50-100 %) and gliosis (50-100 % increase of glia) were already evident (Fig 1B) There was no detectable manganese accumulation in CA1 indicated by lack of abnormal hyperintensity in T1 wt images and the T1 profile which stays above 1000 ms in CA1 region. This indicates that 1.5-2 fold increase in gliosis has very small effects on MEMRI contrast.

At 12 days post status epilepticus, gliosis and cell loss were more prominent but axonal sprouting was still very weak (score 1-2 /5) (Fig 1C). The T1 profile shows a decrease in the area corresponding to the stratum radiatum and CA1, which were the regions with most significantly increased gliosis (>2 fold). Thus, it appears that the gliosis starts to play significant role in MEMRI contrast when its amount of glia is more than two fold compared to control brain.

At 2 months there was significant inter-animal variation both in MEMRI and in histological findings. Three cases showing different levels of gliosis, neuronal death and sprouting and different patterns of hippocampal MEMRI enhancement are presented (Fig 1D-F). In the rat presented in fig 1D, significant gliosis was detected only in middle CA1 (square in figure) while MEMRI showed clear enhancement in the entire CA1 region. Furthermore, profound mossy fiber sprouting was detected in DG. It is likely that sprouting in the DG is associated with sprouting in other subregions of hippocampus, a phenomenon that is not detected by Timm staining but is known to take place in epileptogenic animals [6]. Therefore, it seems plausible that axonal sprouting contributes to MEMRI contrast also after systemic injection of Mn. In Fig 1E, a rat with substantial gliosis (up to 10-fold increase), severe neurodegeneration and only very mild sprouting is shown. In this case all MEMRI changes can be explained in terms of massive gliosis, including enhancement of CA1 and thickening of CA3b. In addition, a few cases with uneven MEMRI enhancement of CA1 were detected (Fig 1F). In this case T1 wt hyperintensity was co-localized with significant neuronal loss and mild to moderate gliosis. Sprouting was in the level of control animals in DG. Our data show that the decreasing neural density, increasing glial density, and increasing axonal density have competing parallel effects on the accumulation of manganese after systemic Mn injection. When gliosis is present in excessive amounts it will most likely dominate the MEMRI contrast. However, it seems that sprouting can also be detected after systemic manganese injection, in cases when contribution of gliosis can be evaluated independently. MEMRI is an excellent tool to detect cellular level changes associated with epileptogenesis. It remains to be seen whether these individual contributions could be separated.

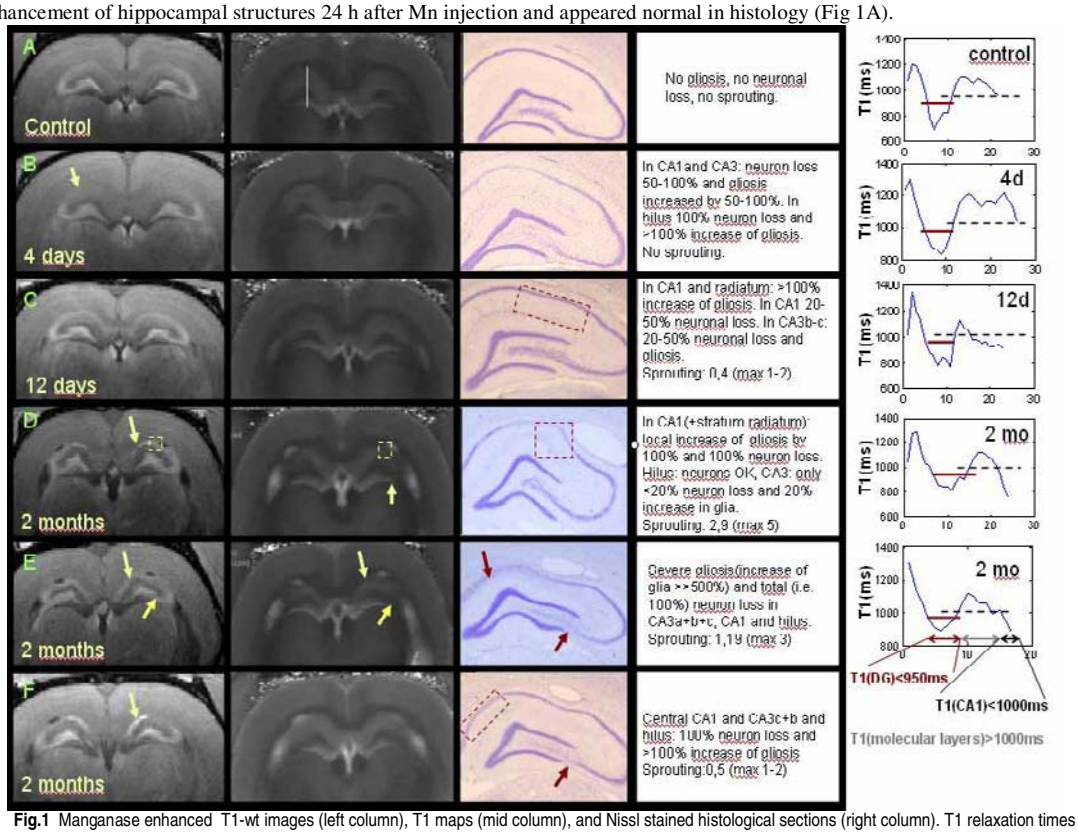

Fig.1 Manganase enhanced T1-wt images (left column), T1 maps (mid column), and Nissl stained histological sections (right column). T1 relaxation times accross the hippocampus (see line) are shown on the right. Rows represent animals imaged at different time points after status epilepticus.

References: [1]Silva, Korezky review, 2004, [2] Nairismägi et al., 2005, [3] Lee *et al.* 2005, [4]. ISMRM 2006 proceedings, [5]Cavazos et al. 1991, [6](Smith and Dudek, 2002

Supported by the Academy of Finland, Emil Aaltonen Foundation and the Finnish Cultural Foundation of Northern Savo