In Vivo Brain Phenotypes of the Reeler Mutant Mouse by using DT-MRI and MEMRI

L-A. Harsan[1], C. David[2], D. Paul[1], S. Schnell[1], J. Hennig[1], J. F. Staiger[2], and D. von Elverfeldt[1]

[1]Diagnostic Radiology, Medical Physics, University Hospital, Freiburg, Germany, [2]Department of Neuroanatomy, Institute for Anatomy and Cell Biology, Center for Neuroscience, Freiburg, Germany

Introduction:

The neurodevelopmental signaling protein reelin controls the neuronal migration and the lamination of several structures of the brain, including the neocortex and the cerebellum. Mutations in the gene encoding the reelin protein induce severe disorders of the central nervous system, in humans (lissencephaly, cerebellar hypoplasia) and in mice. The *Reeler* mutant mouse (mutation in the *reelin* gene) is a well known animal model, characterized by ataxia, hipotonia and fine tremors caused by the disruption of neuronal layers in the brain. The abnormalities in the cellular migration lead to distortions in the dendritic trees and axonal projections, especially in cortical structures. Since these neurodevelopemental alterations show similarities with certain aspects observed in schizophrenia, autism and lissencephaly, the model become a preclinical model for these disorders. The primary goal of the present study was to characterize *in vivo* and non-invasively the *Reeler* mouse brain by analyzing the brain morphology as well as the brain connectivity. For this purpose several magnetic resonance imaging methods, including manganese enhanced imaging (MEMRI) and diffusion tensor magnetic resonance imaging (DT-MRI) were used. MEMRI aimed to provide a comprehensive insight into the neuronal layers organization in several *Reeler* brain structures, such as hippocampus or cerebellum. DT-MRI and the fiber tracking procedures were designed to be comparable with those used for human studies and aimed to reveal the reorganization of neural pathways in such pathological conditions. Furthermore, the study explored the use of probability mapping to determine – in a statistical sense – all possible connecting pathways between different regions of interest. The connection pathways obtained in vivo were validated using histology based tracing methods.

Materials and Methods:

Seven wild-type B6C3Fe adult female mice and 7 *Reeler* mutant mice were scanned using a 9.4T small bore animal Scanner (Biospec 94/20, Bruker, Ettlingen, Germany). DT-MRI data was acquired in 31 axial slices (156 x 156 x 500 µm) using a 4-shots DT-EPI sequence. Diffusion gradients were applied in 30 non-collinear directions for a b factor of 1000s/mm^2. The diffusion tensor was calculated using an in house developed DT-MRI software(1). Different diffusion tensor parametric maps were generated, including fractional anisotropy (FA), and mean diffusivity <D> as well as directional encoded images (i.e. Fig. 2).

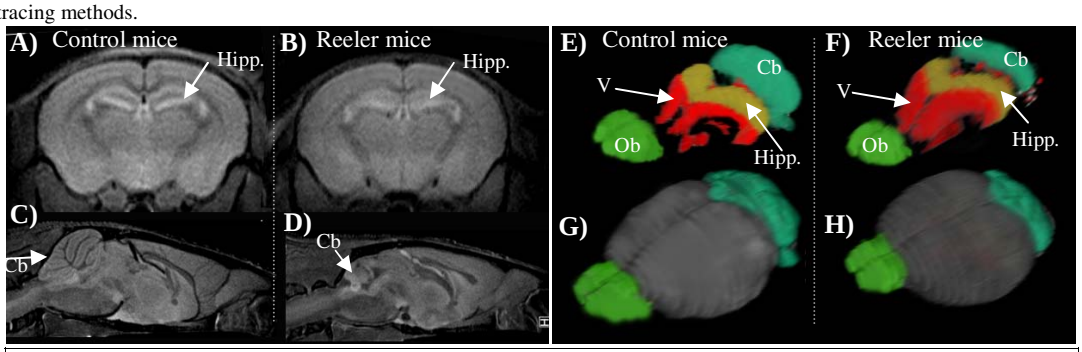

Figure 1: A,B,C,D. MEMRI of wild –type (A,C) and Reeler brains (B,D). Notice the specific enhancement of hippocampal layers (A – Hipp.) in wild type brains and the diffuse signal in disorganized hippocampus of the Reeler mice (B-Hipp.). The cerebellar hypoplasia and the absence of cerebellar foliations is observed in the Reeler brain (D). **E,F,G,H.** 3D reconstruction of wild-type and Reeler brains. Smaller olfactory bulb (Ob), hippocampus (Hipp.), and cerebellum (Cb) were measured in Reeler mice. The ventricles (V) size was greatly increased in mutants.

Estimates of the axonal fiber projections were computed by the fiber assignment by continuous tracking (FACT) algorithm. Several seed points were further chosen to track the specific connections that we wanted to evidence. A DT-MRI probabilistic approach (2) was additionally used, capable to determine in a statistical way the most probable neuronal pathway connecting two seed regions. Thalamocortical connectivity obtained *in vivo* using DT-MRI was compared with the histological based tracing performed in the same mice after the MRI exam. Following the DT-MRI acquisition the mice were scanned using a RARE-T1 (31 axial slices/500µm thickness) TR/TE=1500ms/7.5ms, 78 x 78 µm in plane resolution) and a RARE-T2 weighted sequence (31 axial slices, 500µm slice thickness TR/TE=4500ms/30ms, 78 x 78 µm in plane resolution). The mice were further ip injected with 120mg/kg $MnCl_2$ solution (120mM) and rescanned 24 hours later with the same RARE-T1 / -T2 protocols.

Results and Discussion:

In the present study, the cytoarchitecture of the *Reeler* brain was visualized by using MEMRI while the brain connectivity (hodology) was depicted *in vivo* and non-invasively by DT-MRI. Disorganization of the neuronal layers is clearly visible in different structures of the *Reeler* brain (see the hippocampus and cerebellum in the Fig. 1- B, D). The various MR based contrasts allowed the brain segmentation, the calculation and the 3D reconstruction (Fig. 1) of the brain volumes. The results showed a great enlargement of the ventricles in Reeler brains (Fig. 1 – F), while the cerebellum, the hippocampus and the olfactory bulb volumes were decreased with 59%, 18% and 16% respectively. In-vivo DT-MRI and fiber tracking procedures showed distorted axonal pathways when compared to wild type brain hodology. The fiber tracking pictorials of *Reeler* brain (Fig. 2, B) show poorly compacted thalamic axonal projections that penetrate the cortical plate and run up diagonally in the outer regions of the cortex. From these areas they descend to the deeper cortical planes (Fig 2. H) where the target neurons wrongly end up their migration. Comparatively, the normal architecture of the thalamocortical projections in wild type mice is exemplified in the Fig. 2 (A). In this case the fibers from the thalamus are crossing the internal capsule, they run tangentially at the interface of the cortex with the subcortical white matter and ascend to rich the target fields more superficially into the cortex. Several other axonal projections embedded into the gray matter or having long and complicated 3D course (i.e. lateral olfactory pathways) were examined in vivo and non-invasively in the Reeler brain and were reconstructed using fiber tracking procedures. Additionally, probability maps of connectivity, able to investigate – in a statistical sense – all possible pathways between seed points and to depict the most likely connections were generated. The method has been demonstrated to be able to extract and quantify neuronal pathways connecting defined small regions and without having *a priori* knowledge about the course of these connections. Furthermore, color coded maps evidenced abnormalities at the level of the cingulum structure (Fig. 2 F-Cg vs C-Cg) in *Reeler* mice. The cingulum is a very important part of the limbic system. In humans, disruption of the limbic system network integrity, in particular the cingulum, was associated with the pathophysiology of the schizophrenia. The failure in the cerebellar layers formation is also visible in color coded maps of the *Reeler* cerebellum (Fig. 2, G,H) and contrasted with the great lamination of the wild type cerebellar cortex (Fig. 2 – D, E). The combination of different MR methodologies (MEMRI, DT-MRI and fiber tracking, brain volumetry) provided a fine *in vivo* description of the *Reeler* mutant brain. The identified brain phenotypes are in good agreement with the results obtained by using histology based procedures.

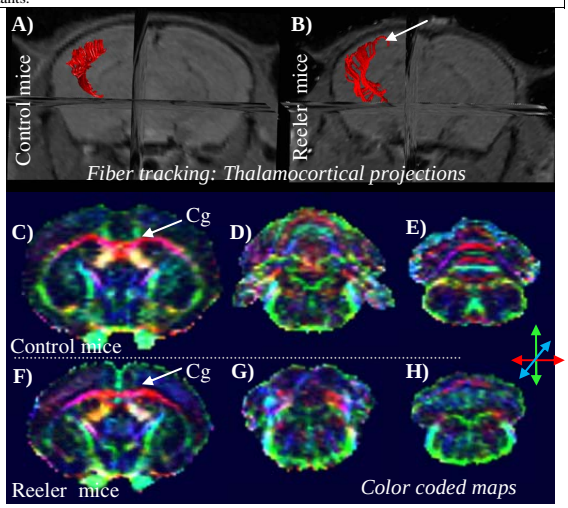

Figure 2: A, B. Thalamocortical projections of the wild-type and Reeler mice. Comparison of the fibers connecting the ventro-basal thalamic nuclei and the somatosensory cortex of a wild type (B) and a reeler mutant mouse (A). **C, D, E, F, G, H.** Color coded maps of wild-type and Reeler brains**.** No fiber bundles, comparable to the position of the cingulum could be identified in the mutant brain (F-Cg vs C-Cg). Cerebellar lamination is visible in the wild-type brain (D, E) but not in the reeler mice (G, H).

(1). Kreher et al., Magn Reson Med 2005;54(5):1216-1225; (2) Kreher et al., NeuroImage 2008;43 (1):81-89.