In Vivo Diffusion Tensor Magnetic Resonance Imaging and Fiber Tracking of the Mouse Brain

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Introduction:

Neuronal connectivity studies are of great interest since circuitry is highly correlated to brain function under normal conditions, or to dysfunction under pathological circumstances. Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) has become a reference tool in reconstructing neuronal pathways *in vivo,* especially in human brain. The increasing number of animal models that mimic human brain disorders offers the potential for the use of this method in analyzing axonal tracts and characterizing brain microstructure. A close comparison and correlation between DT-MRI data sets obtained in patients with brain disorders and brain imaging data of the corresponding mouse models require the use of comparable acquisition schemes in terms of spatial and angular resolution. The primary purpose of this work was develop a DT-MRI protocol for *in vivo* mouse brain imaging, able to provide comprehensive insight into the mouse brain connectivity, as seen in human studies. Improvement of the angular sensitivity aimed to facilitate the fiber tracking of the major white matter tracts (corpus callosum, anterior commissure, fimbria-fornix complex) and to detect more subtle and three dimensionally complex connections that have not been visualized so far *in vivo* in the mouse brain. Data analysis focused on the possibility to investigate brain regions belonging to the limbic system (amygdala) and its complex connections (amygdala – bed nucleus of stria terminalis, amygdala – cortex), being very important in studies related to depression, fear or addiction behavior in rodents. Furthermore the study aimed to explore the use of probability mapping to determine – in a statistical sense – all possible connecting pathways different regions of interest. A first test performed on the reeler mutant mouse, model of cortical development disorders was designed to prove the method potential for characterizing *in vivo* the impaired thalamocortical connectivity.

Materials and Methods:

Seven wild-type C57BL/6 adult female mice and a reeler mutant mouse 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 of 500µm thickness using a 4-shots DT-EPI sequence. The in plane image resolution was of 156 x 156 µm at a FOV=20 x 20 mm. 6 averages were used to increase the signal to noise ratio. The brain axial slices were acquired over 91 minutes for each mouse, with diffusion gradients applied in 30 non-collinear directions and a b factor of 1000s/mm^2. Navigator echoes were employed to correct for possible ghost induced artifacts. The diffusion tensor was calculated using an in house developed DT-MRI post-processing tool (1). Different diffusion tensor parametric maps were generated, including FA, VR and mean diffusivity <D> as well as directional encoded images. 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.

Results and Discussion:

In the present study, different types of prominent and less prominent mouse brain fiber pathways were identified and displayed three dimensionally using *in vivo* non-invasive DT-MRI. The major WM tracts were rendered in 3D pictorials, with a high angular resolution derived from the use of 30 diffusion gradient directions for data acquisition. Several axonal projections embedded into the gray matter or having long and complicated 3D course (stria terminalis; thalamocortical projections, amygdala – cortex) were visualized for the first time *in vivo* and non-invasively in the rodent brain and were displayed using fiber tracking procedures (Fig.1, A, B) and probability maps of connectivity. The latter procedure investigates – in a statistical sense – all possible pathways between two seed points and depicts the most likely connections. Our method has thus 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 A first test performed on one reeler mutant mouse proved the potential of the described methodology for application studies in animal models of brain disorders. Both, the FACT algorithm (Fig.1, C, D) and the probabilistic approach (Fig. 1, E, F) allowed the *in vivo*, non-invasive identification of the abnormal fiber connectivity between the ventral posteromedial thalamic nucleus (VPM) and the barrel field from the primary somatosensory cortex (S1BF) of the reeler mutant mouse.

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

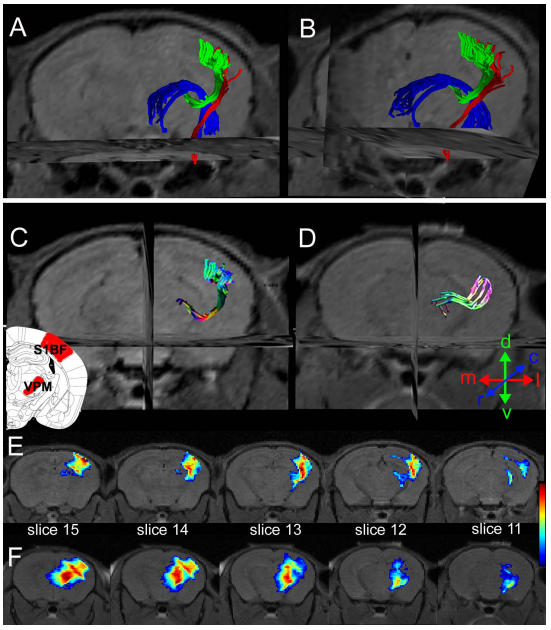

Figure 1: A, B. Assembly of three important brain fiber tracts of a wild-type mouse. Blue fibers: stria terminalis; green fibers: thalamocortical projections; red fibers: amygdala - cortex

C, D. Comparison of the fibers connecting the ventral posteromedial thalamic nucleus (VPM) and the barrel field from the primary somatosensory cortex (S1BF) of a wild type (C) and a reeler mutant mouse (D). Abnormal projections of the thalamocortical axons of the reeler mutant brain are observed in fiber tracking pictorials (D) and in probability maps of connectivity (F), when compared to the wild-type brain mapping (C, E).