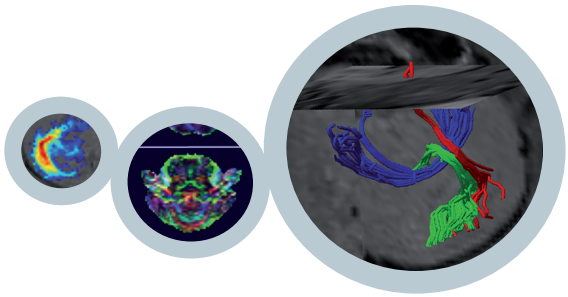
Bruker BioSpin MRI



In vivo insight in the mouse brain tissue microstructure and connectivity by DT-MRI and Fiber Tracking

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Until very recently, the study of neural architecture and its integrity in animal models was exclusively done by using histology-based imaging modalities applied to fixed brain tissue. A non-invasive detailed insight into the brain's microsctructure and axonal connectivity *in vivo* has only become possible since the development of diffusion tensor magnetic resonance imaging (DT-MRI) [1].

The technique relies on the phenomenon of diffusion of water molecules in tissue. For each voxel of the image the diffusion tensor describes the magnitude and directionality (anisotropy) of the water movement [2], providing an unique composite contrast that reflects the tissue characteristics [3]. Tractographic algorithms are using this information to simultaneously delineate multiple fiber tracts three-dimensionally and to identify alterations in the brain structural connectivity.

Such brain connectivity studies in small animals are challenging but achievable now using developments in the MRI technologies and post-processing algorithms [4,5]. The primary technical challenge for obtaining *in vivo* DT-MRI of the mouse brain is to obtain good spatial resolution while preserving satisfactory signal to noise ratio (SNR), in a reasonable acquisition time. A robust estimation of the diffusion tensor involves also the use of acquisition schemes with high number of gradient encoding directions, prolonging the scanning times and therefore the animal anesthesia. This represents a major limitation especially when studying longitudinally animal models of different pathologies.

The use of high field magnets for rodent brain DT-MRI affords an improved SNR and consequently a better spatial resolution in a shorter acquisition time. In our studies we show the feasibility of *in vivo* DT-MRI and fiber tracking of the mouse brain [4] by using a diffusion weighted Spin Echo (SE) 4-shot echo-planar imaging (EPI) acquisition sequence. The measurement procedure is adapted to mouse brain imaging at 9.4 T, in an acquisition time maintained between 91 and 120 minutes for 30 to 45 diffusion gradient directions. This type of data is suitable for performing the reconstruction of white and gray matter pathways in the living mouse brain, using deterministic and probabilistic tractography. The potential of DT-MRI methodology to quantitatively assess the myelin and the axonal pathology in the cuprizone mouse, an animal model of white matter disorders is also illustrated.

Experimental Protocol:

Magnets & Coils: Imaging is performed with a Bruker Biospec 9.4 T small bore animal system, equipped with a BGA12S gradients. A transmit/receive ^{1}H mouse quadrature birdcage resonator (35 mm inner diameter) and the ParaVision 5 software (Bruker BioSpin MRI) is also used for data acquisition.

Shimming procedure: The magnetic field homogeneity is optimized by performing a localized shimming procedure on a volume of interest (4.8 x 5.3 x 9) mm^3 placed inside the mouse brain, using PRESS waterline spectroscopy protocol and FastMap procedures provided with Bruker ParaVision 5.

Sequence parameters: 4-shot DT-EPI sequence with navigator echos that limit the distortion artifacts. Up to 31 adjacent axial slices of 500 µm in thickness are acquired with a repetition time (TR) of 7750 ms, an echo time (TE) of 20 ms, time (Δ) between the application of diffusion gradient pulses of 10 or 17 ms, diffusion gradient duration (δ) of 4 or 7 ms, depending on the application. 6 averages are used to increase the signal to noise ratio (SNR). The in-plane image resolution is 156 x 217 µm at a FOV=20 x 20 mm and an acquisition matrix of 128x92. Partial Fourier with an acceleration factor of 1.35 and 31 overscan lines are used. The acquisitions are done with two encoding b factors of 0 (b0 image) and 1000 s/mm^2 and diffusion-sensitizing gradients applied along 30 - up to - 45 isotropic directions of three dimensional space. The acquisition time is ranging between 91 to 120 minutes for each mouse. Respiratory gating ensures the limitation of movement artifacts.

Anesthesia: The measurements are carried out under isoflurane (3% for induction and ~1.5% for maintenance) mixed with oxygen (1 l/min) anesthesia. The breathing frequency is maintained at of 75-80 breathings per minute in order to avoid the artifacts that could arise from irregular respiratory movements.

Analysis: ParaVision, FiberTool package developed at Uniklinik Freiburg and SPM tools are employed. Different diffusion tensor parametric maps are generated, including fractional anisotropy (FA), volume ratio (VR), mean diffusivity <D> as well as the main diffusivities (λ_1, λ_2, λ_3) [2,6]. Axial (λ_1) and radial [(λ_2 + λ_3)/2] diffusivities, largely employed for ROI analysis [7] are also calculated. The orientation information is visualized through directional encoded images (color coded maps), which were generated by color coding the primary eigenvector (e1) direction (Fig. 3).

Fiber tracking: Streamline and probabilistic fiber tracking algorithms can be employed. FACT algorithm is the most widely used for human studies and can be also applied for tracking the mouse brain. Criteria for terminating the tracking includes an anisotropy threshold decided by the user in accordance with its specific application (i.e. in our studies: Start criteria: FA values > 0.25, Trace values < $1.6x10^{-3}$ mm^2/s; Stop criteria: FA values < 0.15, Trace values < $2x10^{-3}$ mm^2/s) and a maximum stiffness condition (for our experiments 53°).

Probabilistic fiber tracking: The connection probability between different regions of the mouse brain (e.g. ventral posteromedial thalamic nucleus-VPM and the somatosensory cortex-SSC) can be investigated. Seed regions (i.e. VB and SSC) are placed in accordance to their described position within the mouse brain atlas [8]. The method requires two processing steps: In the first step, probability maps are generated separately for each seed region. The number of random walks for the presented examples was set to 1000. The second step consists of combining the previously generated maps to derive the most probable direct pathway between the corresponding seed regions (in the Fig. 2B the most probable pathway between VPM and SSC).

Results and Discussion:

DT-MRI using 4-shots DT-EPI sequence with high number of gradient encoding directions (30 to 45) and localized shimming procedures is an appropriate methodology for studying connection pathways in the living mouse brain. It allows a three-dimensional characterization of the cerebral tissue, being able to capture a large amount of detailed anatomical information in a nondestructive manner. Major white matter tracts can be reconstructed but also fine grained mapping of rich axonal projections crossing gray matter regions can be revealed (Fig. 1). An example is the complex connectivity of the amygdala, the core structure of the limbic system. In wild type animals, stria terminalis is seen as the major output pathway of the amygdala, projecting from its corticomedial division to the bed nucleus of stria terminalis and hypothalamus (Fig 1-blue). Also the cortico-amygdaloid pathways are detectable (Fig. 1-red).

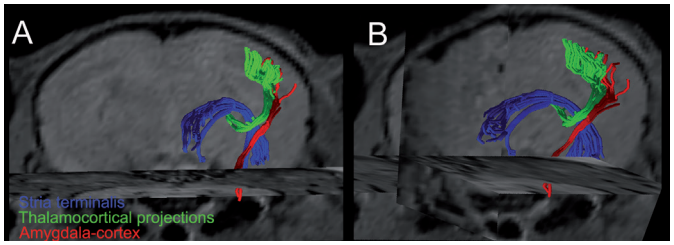

Fig.1: Reconstructed mouse brain fibers from *in vivo* DT-MRI acquisition at 9.4T. **A:** Frontal view, **B:** Lateral view. Blue: stria terminalis. Red: Amygdala-cortex. Green: Thalamocortical projections

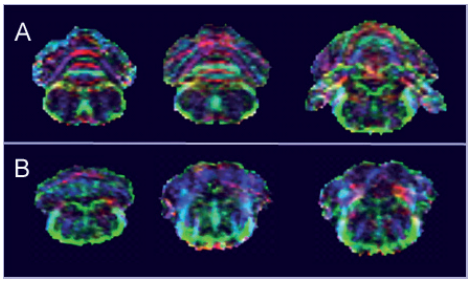

Fig.3: Color coded maps of wild-type and Reeler mouse brains. Cerebellar lamination is visible in the wild-type brain (A) but not in the reeler mice (B)

This opens a new way to investigate the impact of neuropsychiatric conditions such as drug, alcohol addiction, fear or depressive like behavior on connectivity. As in the case of the amygdala, the thalamus, another gray matter region, sends axonal projections to the cerebral cortex. Figure 2 shows the architecture of the thalamocortical projections in wild type mice. Comparatively, the probabilistic tractography (Fig. 2B) identified also the most probable pathway of connectivity between VPM and SSC.

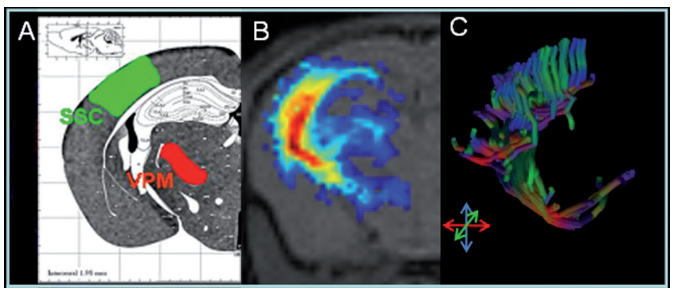

Fig.2: Comparative mouse brain tractography of thalamocortical projections. (A)The seed points were located into the thalamus (VPM) and cortex (SSC). Probabilistic maps of connectivity (B) and FACT generated fiber tracts (C) were obtained after data acquisition using a 4-shot DT-EPI sequence with 30 gradient diffusion directions.

Moreover, the color coded images that can be obtained from the diffusion data provide also morphological information and can be of high value in screening the brain morphology of mutant mice. An example is given in Fig. 3 showing the failure in the cerebellar layers formation in the the *Reeler* mutant mouse, a model of misguided neuronal migration. This is contrasted with the great lamination of the wild type cerebellar cortex imaged *in vivo*.

Beside the orientation information and the structural connectivity, DT-MRI is able to provide images of rotational invariant parameters of the diffusion tensor, such as fractional anisotropy (FA), volume ratio (VR), axial (λ_1), and radial [(λ_2 + λ_3) /2] diffusivity. These parameters are used for quantitative evaluation of microstructural modifications in developmental and pathological conditions. Of great interest is the evaluation of the radial and axial diffusivities derived after the diffusion tensor calculation in the brain of animal models of white matter disorders. Results from our studies and from other groups [7] suggest that increased radial diffusivity could serve as a surrogate marker of demyelination while the reduced values of axial diffusivity are observed in brain areas of axonal damage. The histopathological investigation shows this type of correlation (Fig. 4).

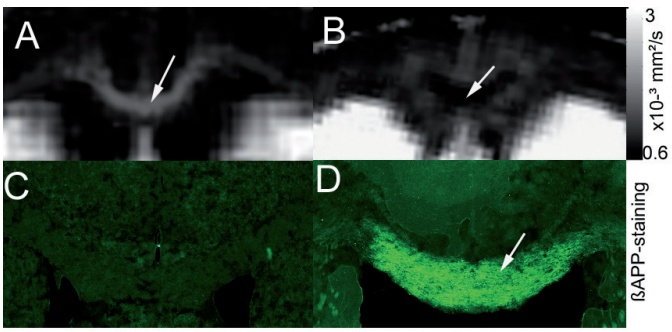

Fig.4: Decreased axial diffusivity in demyelinated mice (B) is correlating with the axonal damage detected trough histopathology (D). Imaging data was acquired at 9.4T using a 4-shot DT-EPI sequence with 45 gradient diffusion directions.

DT-MRI remainins so far the only methodology able to distinguish and evaluate the axonal and myelin damage *in vivo* and non-invasively.

Summary and Outlook

DT-MRI remainins so far the only methodology able to distinguish and evaluate the axonal and myelin damage *in vivo* and non-invasively.

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