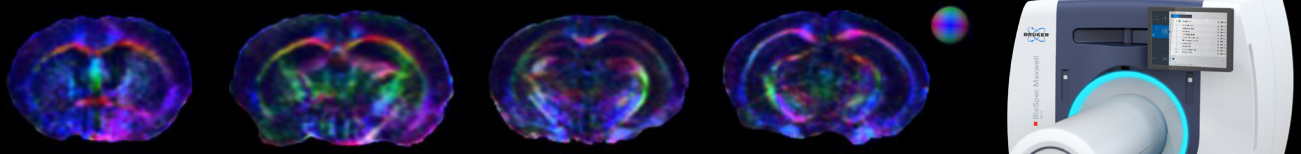

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Preclinical MRI of neurological diseases – from morphological imaging to the mapping of tissue properties

Jan Klohs and Sarah Herrmann

Innovation with Integrity

The role of clinical and preclinical imaging in neurological disease studies

In neurological diseases, damage to the central nervous system can arise from congenital abnormalities or genetic disorders, or result from ischemic, traumatic, or non-traumatic injury, infections, autoimmune responses, or from lifestyle and environmental factors. Pathological changes at the molecular and cellular level cause functional and cognitive impairments and pose a massive clinical, social, and economic burden.[1] With increasing life expectancies, the incidence of age-related diseases such as stroke and Alzheimer`s disease, that are leading causes of death and disability, is rapidly increasing worldwide. Despite major efforts, the number of effective therapies that exist is very limited.

Magnetic resonance imaging (MRI) is an established modality in the management of patients with neurological diseases. In conjunction with neurological assessments, anatomical MRI has improved clinical diagnosis, and treatment stratification and monitoring. Moreover, MRI is an important research tool in drug development and the study of pathophysiology of neurological diseases. The preclinical MRI research field influences modern clinical MRI by providing and validating novel tools for diagnosis and monitoring. Using animal models of neurological disease, preclinical MRI gives valuable insights into the complex pathophysiology of neurological diseases as well as the evaluation of novel therapeutic interventions and regenerative mechanisms.

A variety of species are used for preclinical MRI including insects, fish, and birds. However, rodents are most commonly used as their nervous systems have a similar organization and complexity to those of humans. Disease can be recapitulated by experimental manipulations (occlusion of a blood vessels, contusion of the brain or spinal cord, etc.), transgenic engineering, or by altering diets and environmental conditions. Owing to their relatively short life span rodents are suitable models for longitudinal and interventional studies of age-related diseases.

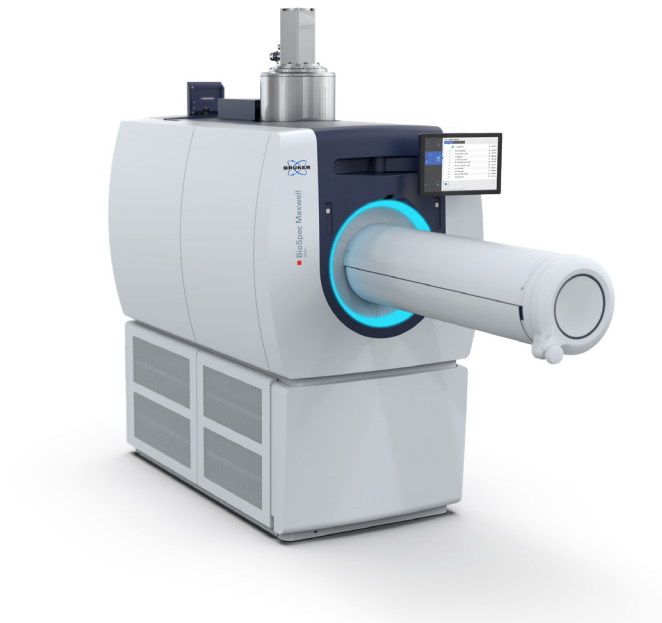

Figure 1: BioSpec Maxwell. BioSpec Maxwell MRIs are compact liquid cryogen filling-free MRI instruments available at field strengths of 3 Tesla, 7 Tesla, and 9.4 Tesla.

Tailoring MRI for preclinical neurological research

Many clinical MRI techniques can be applied in preclinical studies, however, preclinical MRI requires dedicated hardware tailored to address its inherent challenges. The small dimensions of mouse and rat central nervous systems require smaller voxels and higher sensitivity to obtain MRI data with resolution and signal-to-noise ratio that is comparable to that of human clinical scans. While 3 Tesla and 7 Tesla preclinical MRI instruments are available and can have an advantage for translational neurological studies, MR instruments operating at 9.4 Tesla or even higher field strengths offer significant gains in signal-to-noise ratio and use of higher acceleration factors for faster scanning.[2]

In addition, strong gradients with high slew rates, high duty cycles, and high-power amplifiers enable fast acquisition of small volumes in animal nervous systems. Dedicated surface array coils yield highest sensitivity for areas of interest that are closest to the coil, such as is the case for brain and spinal cord imaging for which they provide ideal coverage. As cryogenic radiofrequency coils, they provide an even additional boost in sensitivity.[3] The gains in signal-to-noise ratio can either be invested in obtaining images with higher spatial or temporal resolution or for shortening the acquisition time of scans, thus allowing to obtain multiple read-outs during an MRI examination, to reduce the exposure time of an individual animal, or to enable higher throughput.

While generally MRI of awake animals is possible and even desirable in some studies, animals are often anesthetized to minimize movements during scanning. This necessitates the installation of equipment for supplying anesthesia within the MRI system together with systems that monitor the physiological function of the animal under study. This physiological information feedback is often implemented into scanning protocols.

The latest developments in preclinical MRI technology are combined in Bruker´s BioSpec Maxwell series of 3 Tesla, 7 Tesla, and 9.4 Tesla field strength instruments which require no liquid helium filling **(Fig. 1)**. Along with the MRI CryoProbe, volume and surface coils, which are specifically designed for brain and spinal cord imaging are available for these instruments **(Fig. 2)**. Bruker's ParaVision software provides pre-optimized protocols for common preclinical species and applications studies, as well as a software framework for MR method development and execution. The software suite accompanies the planning and acquisition of scans, to obtain consistent and reliable data and its viewing, reconstruction, and analysis tools yield the most information from the obtained imaging data.

Figure 2: BioSpec Maxwell portfolio of dedicated RF coils. **A)** With up to 82 mm inner diameter, the range of volume coils for BioSpec Maxwell instruments accommodates preclinical imaging species ranging from mice up to large rats. **B)** Dedicated brain and spine coils are optimized to ideally match anatomical regions of interest. **C)** For studies requiring highest resolution the MRI CryoProbe provides a sensitivity boost.

Assessing morphological and macrostructural changes

MRI is ideally suited to assess tissue changes related to neurological diseases at different temporal or spatial scales. While in some processes, like acute trauma or ischemia, gross anatomical changes can be readily assessed within minutes after the insult, in other neurodegenerative disorders pathological changes occur over months and even years, requiring repetitive assessments to detect gradual tissue changes. Due to the non-invasive nature of MRI, studies can be conducted in which the measurement technique does not affect the animal's health state, making non-biased longitudinal studies possible.

With the use of extremely stable instruments, macrostructural information of the brain and spinal cord can be rapidly acquired or if desired remarkably high-resolution scans obtained over extended time periods (**Fig. 3**). Different MRI methods (T1, T2, proton density, etc.) are used to obtain anatomical contrast between the gray and white matter and cerebrospinal fluid (**Fig. 3A**), and to detect administered contrast agents. Structural MRI allows to assess gross morphological changes (e.g. atrophy) in the brain and spinal cord in acute and longitudinal evaluations, while acquisition with high resolution can capture morphological changes of anatomical substructures (e.g. substantia nigra, optic nerve). In addition, anatomical contrast allows determination of the location, extent, and shape of an acute tissue damage or tumor, and enables detection of edema and hemorrhages as well as smaller structural abnormalities like microbleeds, calcifications, or metastasis. *Ex vivo* structural imaging of fixed specimens with very high spatial resolution obtained using long data acquisitions provides complement information about the cytoarchitecture of regions or assess areas that are more challenging to image *in vivo* (**Fig. 3B, C**).

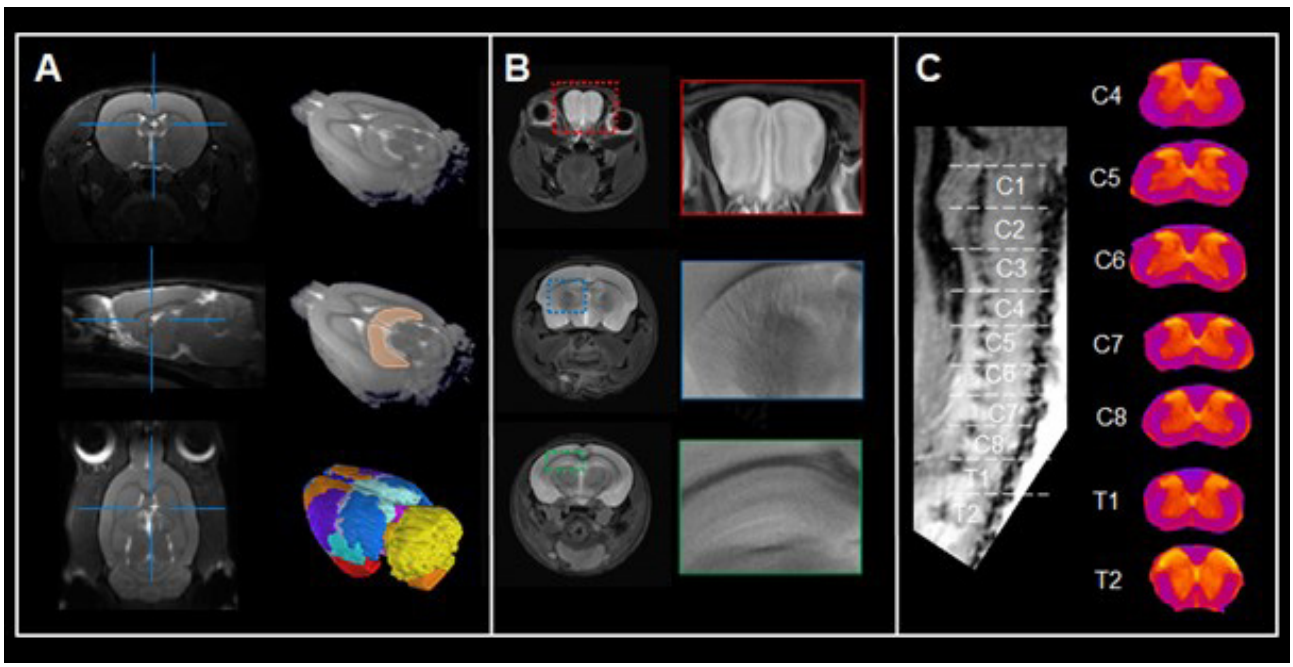

Figure 3: Morphological and macrostructural MRI. **A)** High resolution T2-weighted images of a rat brain in axial, sagittal and coronal view and 3D renderings with segmentation. Images allow to identify anatomical regions and to assess morphology of the brain. **B)** High resolution T2-weighted *ex vivo* images (26 µm x 26 µm in plane resolution) of a fixed mouse brain. Zoom-ins depict the olfactory bulb (red box), striatum (blue box) and hippocampus (green box). **C)** *Ex vivo* scans of a mouse spinal cord, showing axial images of different spinal segments. All data were acquired with a BioSpec Maxwell 94/17. Data for **A)** and **C)** were acquired using a volume coil for transmission and a phased-array coil for reception, while data for **B)** were acquired with a transmit-receive volume coil. T2-weighted images were acquired with TurboRARE sequences with different sequence parameters. Data visualization and segmentation with ParaVision 360 and PMOD.

Structural imaging can be used in therapeutic studies for stratification, and monitoring treatment response (change in lesion or tumor size, atrophy rates, etc.). Structural imaging can also guide the administration of novel therapies such as viral vectors, genes, antibodies, and growth factors to their target site within the central nervous system. When used for administering therapeutics, it is often combined with approaches to disrupt or circumvent the blood-brain barrier (e.g. focused ultrasound, carrier molecules).

Visualizing pathological changes in specific central nervous system tissues

In some neurological diseases pathology extends across the whole brain and spinal cord requiring organ-wide assessment of disease burden, while in other disease conditions the pathology affects only certain tissues, requiring tissue-specific investigations. Special sequences can be used to enhance the visualization of specific tissues (**Fig. 4**), allow for tissue classification and segmentation and thus to provide quantitative information about the tissues. For example, T2-weighted images provide excellent contrast between gray and white matter (**Fig. 4A**). Segmentation of gray and white matter is required for the calculation of tissue volumes. Volumetry can be used for the characterization of developmental abnormalities or degenerative changes.

In comparison, the TrueFISP sequence can be used to highlight cerebrospinal fluid spaces (**Fig. 4B**), which are important for production and transport of cerebrospinal fluid in the brain. Altered ventricle volumes or morphology are found in congenital and developmental neurological diseases (e.g. hydrocephalus). Changes to ventricles can also occur in the developed brain in response to neuronal atrophy or injury. Visualization of ventricles can guide intrathecal administration of drugs.

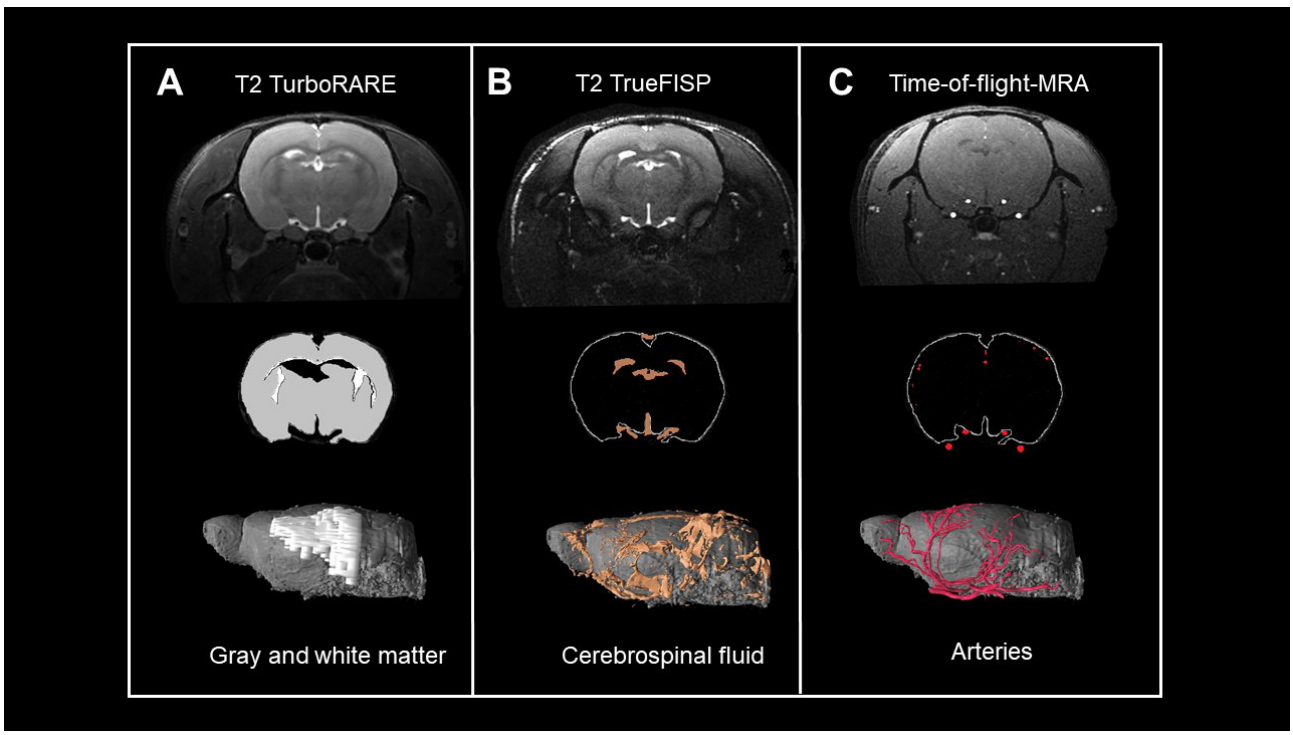

Figure 4: Assessing brain pathology in different tissue compartments Specialized MRI techniques are used to provide tissue-specific contrast of the brain and allow for tissue classification and segmentation such as gray and white matter, cerebrospinal fluid, and arteries. **A)** Gray and white matter (corpus callosum) are visualized with T2-weighted TurboRARE **B)** Cerebrospinal fluid spaces from T2-weighted TrueFISP images. **C)** Arteries from time-of-flight MRA data. Rat brain data was acquired with a BioSpec Maxwell 94/17 using a volume coil for transmission and a phased-array coil for reception. Tissues were segmented and rendered using PMOD.

Methods to visualize blood vessels are also of importance, as alterations of blood vessels have been implicated in a variety of neurological diseases. Time-of-flight MR angiography (MRA) and phase contrast angiography can visualize larger vessels in the brain (**Fig. 4C**) and spinal cord, while contrast-enhanced angiography also allows assessment of finer vessels. Three-dimensional renderings of angiograms (**Fig. 4C**) allow to assess density, distality, and morphology of vessels and thus to assess vascular malformations and remodeling (e.g. anastomosis, aneurysm), stenosis, or occlusions in congenital and acquired neurological diseases.

Quantifying alterations in tissue microstructure and composition

Understanding of the diseased central nervous system crucially depends on reliable knowledge of its composition and its microstructural and cellular organization. Many neurological diseases are characterized by early subtle changes at the microscopic level without apparent gross anatomical changes. Quantitative MRI provides voxel-wise quantifiable measures of specific tissue parameters such as magnetization transfer, magnetic susceptibility, relaxation times or rates, and diffusion parameters that are related to microstructural features (demyelination, axon loss, etc.) and the composition of the tissue (iron, macromolecules, myelin content, ß-amyloid accumulation etc.). Thus, these metrics can provide information about the underlying degenerative and regenerative changes after an acute injury or during chronic progression. For example, de- and remyelination may be reflected by changes in the T1 relaxation time, magnetization transfer, and magnetic susceptibility of the white matter. Parametric maps of the relaxation rates of a rat brain *in vivo* are depicted in **Fig. 5A**. Quantitative MRI can yield more sensitive and specific measures of tissue changes than structural and morphological read-outs. It can be used to track the dynamics of tissue changes in longitudinal studies and provide metrics of treatment response, thus providing MRI biomarkers of neurological diseases.

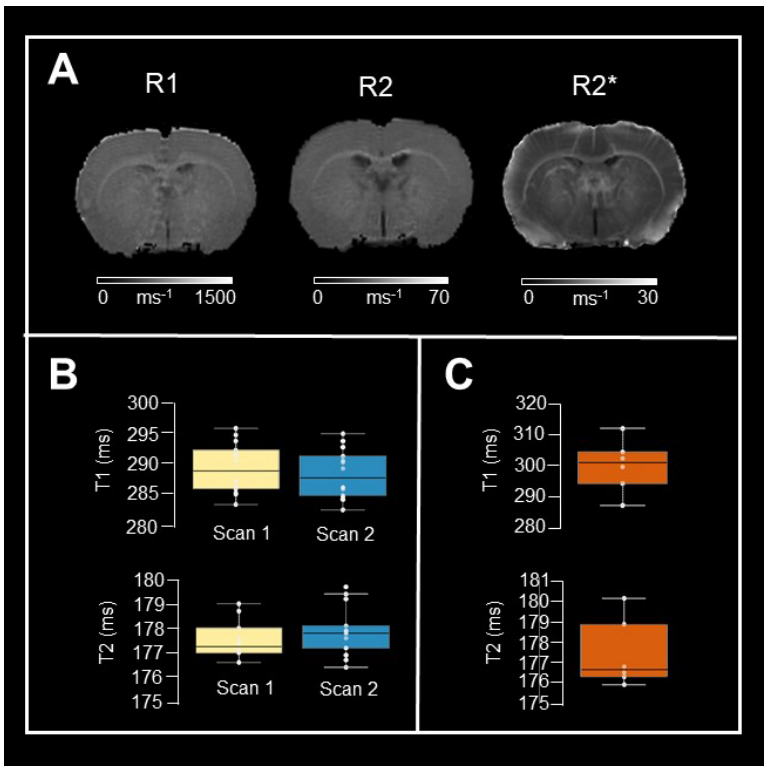

Figure 5: Relaxometry for examination of tissue composition. **A)** Axial R1, R2 and R2* maps of a rat brain. **B)** Test-retest (n = 15) of T1 and T2 with a standardized Bruker liquid phantom to assess repeatability. The phantom was measured twice consecutively while being placed in the instrument. **C)** To assess reproducibility, the Bruker liquid phantom was measured repeatedly seven times within a time interval of 19 days. Both rat brain and phantom data were acquired with a BioSpec Maxwell 94/17 using a volume coil for transmission and a phased-array coil for reception. Box plots were made with R software. The center lines show the medians, box limits indicate the 25th and 75th percentiles, the whiskers extend 1.5 times the interquartile range from the 25th and 75th.

A vital aspect of the development of quantitative biomarkers is their repeatability and reproducibility. Variability in values can arise from variations in scanner performance due to instrument instabilities or variations in the measurement process. Manufacturer tests have shown in test-retest and repeated measurements that relaxometry with the BioSpec Maxwell 94/17 has an excellent repeatability. For test-retest measurements T1 and T2 mapping with a standardized liquid phantom were performed (**Fig. 5B**). The test measurement yielded a median T1 = 286.9 ms (interquartile range (IQR) = 5.8 ms) and the retest measurement a T1 = 286.1 ms (IQR = 5.5 ms), respectively, with an intraclass correlation coefficient = 0.98 (n = 15). For T2, the test measurement resulted in a median T2 = 177.2 ms (IQR = 0.8 ms) and the re-test measurement gave a T2 = 177.9 ms (IQR = 0.8 ms), respectively, with an intraclass correlation coefficient = 0.86 (n = 15). In addition, reproducibility was assessed by performing seven measurements of T1 and T2, within an interval of 19 days between the first and the last measurement (**Fig. 5C**). The median T1 = 302.3 ms (IQR = 11.1 ms) with a coefficient of variance = 0.7 %. The median T2 = 117.6 ms (IQR = 1.7 ms) with a coefficient of variance T2 = 0.3 %. Thus, the stabile performance of BioSpec Maxwell instruments ensures quantitative MRI parameters in preclinical studies. Implementing measures to improve reproducibility (e.g. defining minimum reporting standards, standardization of acquisition protocols and phantoms) can help to compare research findings between different researchers and imaging sites.

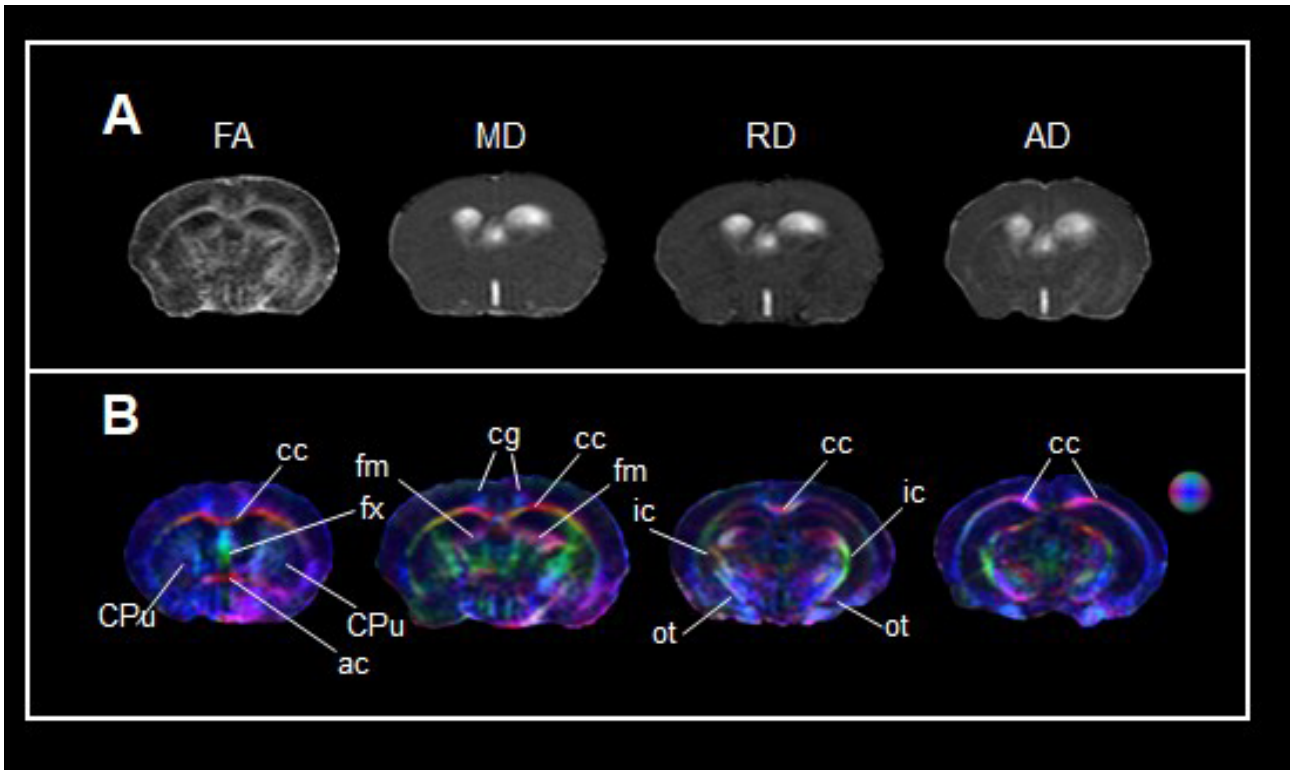

Figure 6: Diffusion tensor imaging for assessing microstructural features. **A)** Axial images of fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) maps computed from DTI data are shown. The parameters give information about the magnitude and directionality of water diffusion. **B)** Axial direction-encoded color maps of the rat brain computed from the same DTI data set. The FA maps, color-coded by the diffusion orientation, are overlaid with the primary diffusion direction. The following white matter structures were identified: Anterior commissure (ac), corpus callosum (cc), cingulum (cg), caudate putamen (CPu), fimbria hippocampus (fm), fornix (fx), internal capsule (ic), and optical tract (ot). DTI data was acquired with a BioSpec Maxwell 94/17 using a volume coil for transmission and a phased-array coil for reception with standard protocols and typical acquisition times. Data post-processing, and visualization with ParaVision 360.

Quantitative MRI based on assessing the magnitude and directionality of diffusion of water molecules via apparent diffusion coefficient or fractional anisotropy is widely used. The apparent diffusion coefficient is a measure of the magnitude of diffusion in tissue. Coefficient maps can be used to delineate lesions or edema and show differences in the cellularity of tumors. The diffusion tensor is used to describe the directionality of water diffusion. An example of *in vivo* diffusion tensor imaging (DTI) maps of a rat brain is shown in **Fig. 6A**. High anisotropy of diffusion is observed in highly oriented tissues like white matter fibers (**Fig. 6A, B**). Changes in fractional anisotropy in the white matter are indicators of degenerative damage as well as regeneration and repair.[4] Furthermore, the technique allows to map the trajectories of single white matter fiber tracts. Changes in focal connectivity can arise from tissue reorganization after an acute injury. A map of all white matter connections (i.e. structural connectome) can further be constructed. Changes in the connectome may identify networks that are vulnerable to a pathology which spreads preferentially over anatomically connected regions. Moreover, advances in biophysical modeling of the diffusion signal enable the characterization of microstructural features (e.g. axon or myelin density), that until now could only be determined using post-mortem histological examinations or invasive techniques.

Conclusion

In summary, preclinical MRI provides a wealth of information on processes involved in neurological diseases either by assessing gross anatomical and morphological changes, or by quantitatively measuring biophysical tissue properties that are related to tissue microstructure and composition. The tools yield spatially and temporally resolved information about disease mechanism, provide validated imaging biomarkers of disease for clinical imaging, and are used for the evaluation of therapeutic approaches.

List of abbreviations

AD = axial diffusivity
DTI = diffusion tensor imaging
FISP = fast imaging with steady state precession
FA = fractional anisotropy
IQR = interquartile range
MD = mean diffusivity
MRA = magnetic resonance angiography
MRI = magnetic resonance imaging
RD = radial diffusivity
RARE = rapid acquisition with relaxation enhancement

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