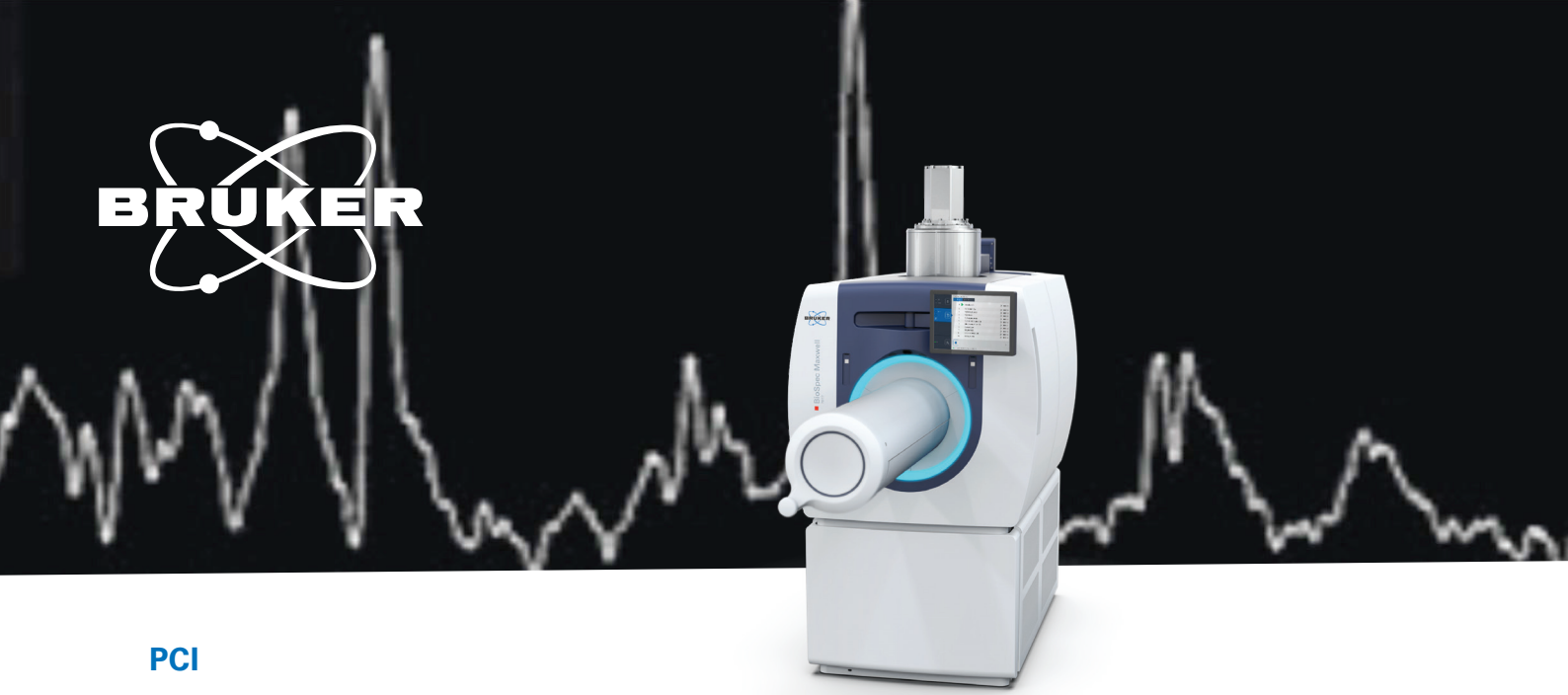

PCI

Preclinical MRI of neurological diseases – functional and metabolic imaging

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Innovation with Integrity

Visualizing pathological processes in the central nervous system with preclinical MRI

Magnetic resonance imaging (MRI) is an established imaging modality in the clinical management of patients with neurological diseases. It provides macro- and microstructural tissue markers, and thus offers, in conjunction with neurological assessments, fundamental guidance for clinical decision-making and outcome prediction. Moreover, MRI is an important mean for research on patients with neurological diseases and in the drug development process. With a growing number of available experimental and transgenic animal models of human neurological diseases, MRI has also become an important tool in preclinical neurological research. The animal models recapitulate vascular, inflammatory, neurodegenerative, traumatic, ischemic, or neoplastic processes and preclinical MRI has been used to characterize the effects of these cellular changes on nervous tissue integrity. Moreover, preclinical MRI supports drug discovery and development, and aids in the validation of clinical imaging findings, thus contributing significantly to the understanding of human neurological diseases.

Dedicated small animal MR instruments have been designed and assembled for the imaging of mice, rats, and other model organisms. The latest advances in preclinical MRI technology development are combined in Bruker`s BioSpec Maxwell series. These Maxwell magnets, which do not require any liquid cryogen filling are available at 3 Tesla, 7 Tesla, and 9.4 Tesla field strengths (**Fig. 1**), all with 17 cm bore diameter, and are equipped with high-performance gradient systems (gradient strength up to 900 mT/m, slew rate = 4200 T/m/s). The three field strengths serve different requirements, enabling translational studies at 3 Tesla and 7 Tesla, and taking advantage of increased signal-to-noise ratios at 9.4 Tesla.

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.

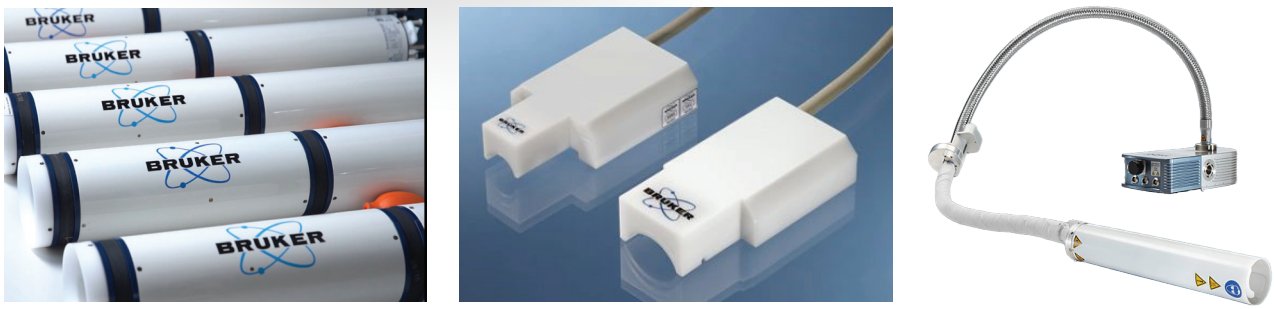

Figure 2: BioSpec Maxwell portfolio of dedicated RF coils. **A)** With up to 82 mm inner diameter, the range of volume coils for BioSpec Maxwell instruments accommodates preclinical imaging species ranging from mice up to large rats. **B)** Dedicated brain and spine coils are optimized to ideally match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

BioSpec Maxwell instruments can be combined with volume and surface coils, well suited for brain and spinal cord imaging **(Fig. 2A, B)**. Cryogenic surface coils provide a significant gain in sensitivity that can be invested in achieving higher spatial resolution or for shortening the acquisition time of scans **(Fig. 2C)**. Bruker's ParaVision provides a software framework for MR method development and execution of pre-optimized protocols for common animal species and applications. The software suite accompanies the planning and acquisition of scans, and its viewing, reconstruction, and analysis tools yield the most information from the obtained imaging data.

MRI acquisition protocols such as T1-weighted or T2-weighted imaging can provide rich anatomical contrast and provide information about the location and extent of central nervous system injury. Moreover, these sequences can capture morphological changes in the brain and spinal cord acutely and over time. Other acquisition protocols can be used to assess specific tissues in the brain and spinal cord. For example, time-of-flight angiography can visualize large blood vessels and thus detect pathological aberrations in the vasculature. Recent research has focused on the development of advanced MRI techniques that yield metabolic and functional information from the central nervous system. These readouts provide more specific measures of central nervous system damage. And as metabolic and functional changes often precede changes in tissue morphology, they might constitute also more sensitive markers of disease.

Monitoring disturbances in energy metabolism and neurotransmitter systems

Metabolic disturbances are a common characteristic of neurodegenerative and inherited diseases and tumors and are a major focus of preclinical neurological research. The methodology of choice for imaging metabolism in the central nervous system is positron emission tomography (PET). PET tracers like [^{18}F]-Fluorodeoxyglucose and [^{15}O]-oxygen are used to quantify regional cerebral metabolic rate of glucose and oxygen metabolism. Bruker's BioSpec Maxwell MR instruments can be combined with a PET inline or PET insert module for sequential or simultaneous PET/MR imaging, respectively and thus used to assess metabolic processes in preclinical models **(Fig. 3)**. Additionally, several MRI techniques also exist to assess metabolic processes in the brain and spinal cord.

Figure 3: Multi-modal PET/MR imaging. PET inline and insert modules are available for sequential or simultaneous PET/MR imaging, enabling multi-parametric investigations.

Magnetic resonance spectroscopy (MRS) implements well-established techniques for quantifying regional biochemistry in the central nervous system.[1] The most widely used approaches are localized ^{1}H MRS acquisition techniques that provide a spectrum of neurotransmitters and metabolites in a region. Two examples of localized ^{1}H MR spectra of the mouse brain acquired with a BioSpec Maxwell 30/17 instrument with a transceive cryogenic quadrature radiofrequency surface probe (MRI CryoProbe) and a BioSpec Maxwell 94/17 instrument with a linearly polarized bird-cage resonator for transmission and phased-array receive-only surface coil in cross-coil mode are shown in **Fig. 4A**. The use of the MRI CryoProbe allows visualization of small molecule resonances even at 3 Tesla, but MRS at 9.4 Tesla yields a ^{1}H spectrum with higher spectral separation and signal-to-noise ratio. The gains results in a largely increased number of detectable metabolites. MRS imaging of multiple voxels allows assessment of neurotransmitter and metabolite concentration variability in different regions. An example of chemical shift imaging of the mouse brain obtained with a BioSpec Maxwell 94/17 in shown in **Fig. 4B**.

Localized ^{1}H MRS and MR spectroscopic imaging can assess regional differences in cell density, cell type, or metabolite concentrations and thus discriminate healthy from pathological tissues. For example, it can characterize lesions of different underlying pathology that have a similar appearance in structural MRI. The use of higher-field MR instruments, robust acquisition techniques, and sophisticated quantification methods allows to obtain functional MRS data with high temporal resolution, thus enabling assessment of pathology-related dynamic changes in neurotransmitters in specific regions. In addition, X-nuclei (e.g. ^{2}H, ^{17}O and ^{31}P) MRS imaging techniques also benefit from use of higher field strengths with regard to sensitivity. For example, in deuterium (^{2}H) metabolic imaging, ^{2}H MRS imaging is performed after the oral intake or intravenous infusion of ^{2}H-labeled substrates and maps of cerebral glucose metabolic rates are generated. The dysfunction of glucose metabolism in neurodegenerative diseases is related to disturbed tissue function and viability, while in contrast, tumor growth is associated with increased glucose metabolism. Other quantitative and noninvasive measurements via X-nuclei MRI are employed in cerebral metabolic rates of oxygen consumption, adenosine triphosphate production, as well as the assessment of intracellular nicotinamide adenine dinucleotide metabolites and redox state.

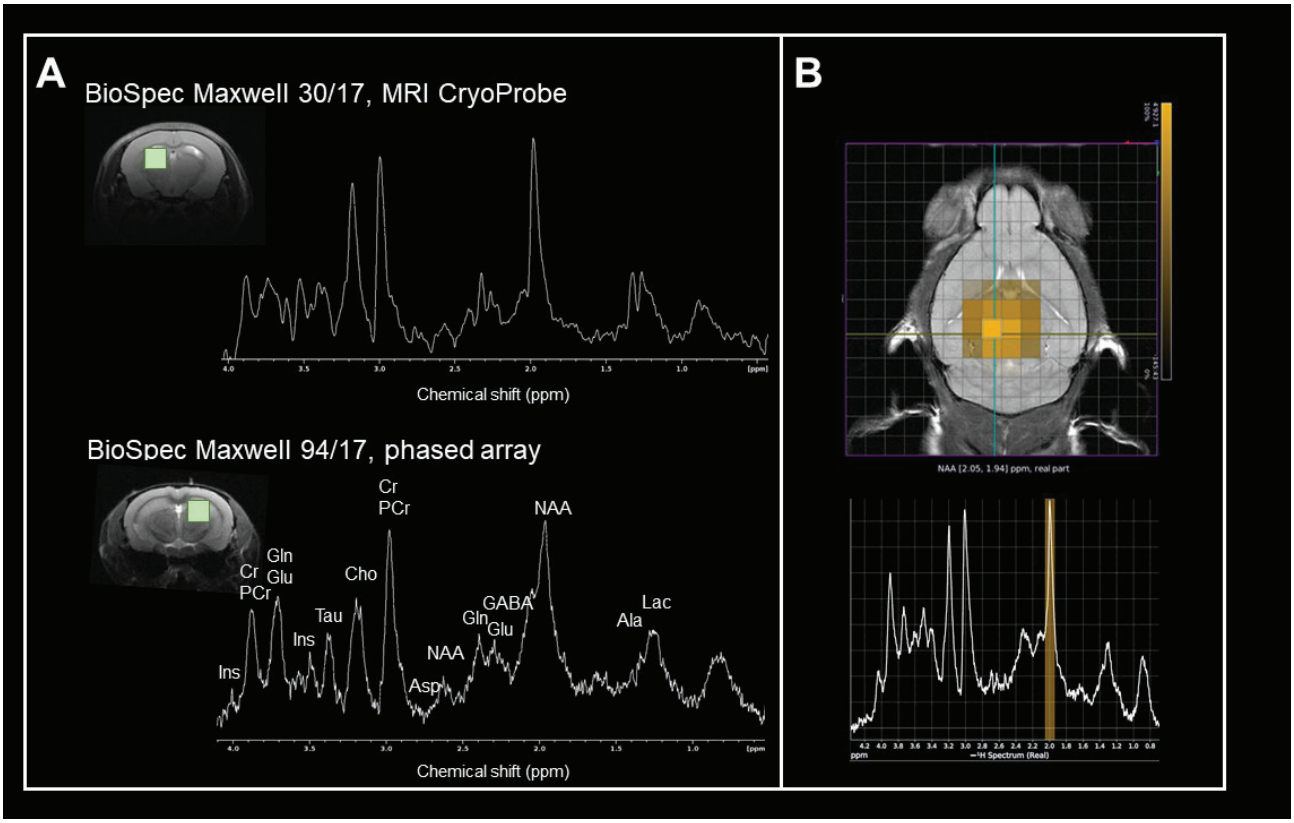

Figure 4. Monitoring levels of neurotransmitters and metabolites. A) Axial FLASH images of the mouse brain with volume of interest and in vivo ^{1}H MRS spectra measured from the corresponding brain regions at 3 Tesla and at 9.4 Tesla. Localization sequence: PRESS, volumes 2 x 2 x 2 mm^3. The following metabolites were assigned: alanine (Ala), aspartate (Asp), choline (Cho), creatine (Cr), γ-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), myo-inositol (Ins), lactate (Lac), N-Acetylaspartate (NAA), phosphocreatine (PCr), and taurine (Tau). Data was acquired with a BioSpec Maxwell 30/17 equipped with a transceive cryogenic quadrature radiofrequency surface probe (MRI CryoProbe) and a BioSpec Maxwell 94/17 using a linearly polarized bird-cage resonator for transmission and a phased-array receive-only surface coil in cross-coil mode. Spectra were processed with TopSpin using the same parameters: line broadening 2, Fourier transform and phase correction. **B)** Chemical shift imaging of the mouse brain. The morphological T2-weighted TurboRARE image shows the position of the CSI grid with a voxel size of 1.25 x 1.25 x 2 mm^3. The corresponding ^{1}H MRS-spectra from the selected voxel are displayed beneath. Data was acquired with a BioSpec Maxwell 94/17 using a linearly polarized bird-cage resonator for transmission and a phased-array receive-only surface coil in cross-coil mode.

An alternative approach to MRS is chemical exchange saturation transfer (CEST) MRI. Compared to MRS, CEST MRI can detect biochemical molecules of interest with higher sensitivity and spatial resolution. In CEST MRI, a radiofrequency pulse is applied to a molecule of interest, which can exchange its ^{1}H protons with those of water at one or more of its resonant frequencies, to reach a saturation state. This magnetic saturation is spontaneously transferred to water via chemical exchange of the excited metabolite protons with non-excited water protons. The subsequent decrease in water signal, can be detected by standard MRI sequences, providing an indirect measure of the concentration of the molecule of interest. Known endogenous CEST molecules typically contain exchangeable groups of amide protons (protein and peptides), amine protons (e.g. glutamate and creatine), and hydroxyl protons (e.g. glycosaminoglycan, myo-inositol). They can be used to study changes in the concentrations of metabolites and neurotransmitters in neurological diseases. Based on metabolic signatures, CEST MRI can be applied for the grading of brain tumors as well as evaluation of tumor therapies. Moreover, some exogenously administered compounds can serve as CEST agents such as glucose and certain drugs which allow to study cerebral and tumor glucose metabolism and to monitor drug actions.

Assessing altered neurofluid patterns and metabolic waste removal

Potential toxic by-products are formed during the metabolic activity of neurons. To maintain homeostasis, the metabolic waste needs to be cleared quickly from the central nervous system. A pivotal route of waste clearance is interstitial and cerebrospinal fluids transport of metabolic solutes. For example, a true fast imaging with steady state precession (True-FISP) sequence gives contrast for cerebrospinal fluid spaces **(Fig. 5A)** and 3D renderings **(Fig. 5B)** allow to assess the anatomy and morphology of the ventricles, which are an important compartment for cerebrospinal fluid production and transport. Altered ventricles are see in number of congenital neurological diseases and in response to neuronal injury or degeneration. Contrast-enhanced, flow and diffusion MRI techniques enable monitoring of interstitial and cerebrospinal fluid flow through different extracellular compartments in the brain and spinal cord. Several neurological diseases display changes in the magnitude and direction of cerebrospinal fluid flow that are associated with an impaired waste removal and accumulation of metabolites (e.g. lactate, ß-amyloid) and trafficking of immune cells. Moreover, assessing neurofluid flow patterns with MRI can help to assess if therapies can restore normal fluid passage.

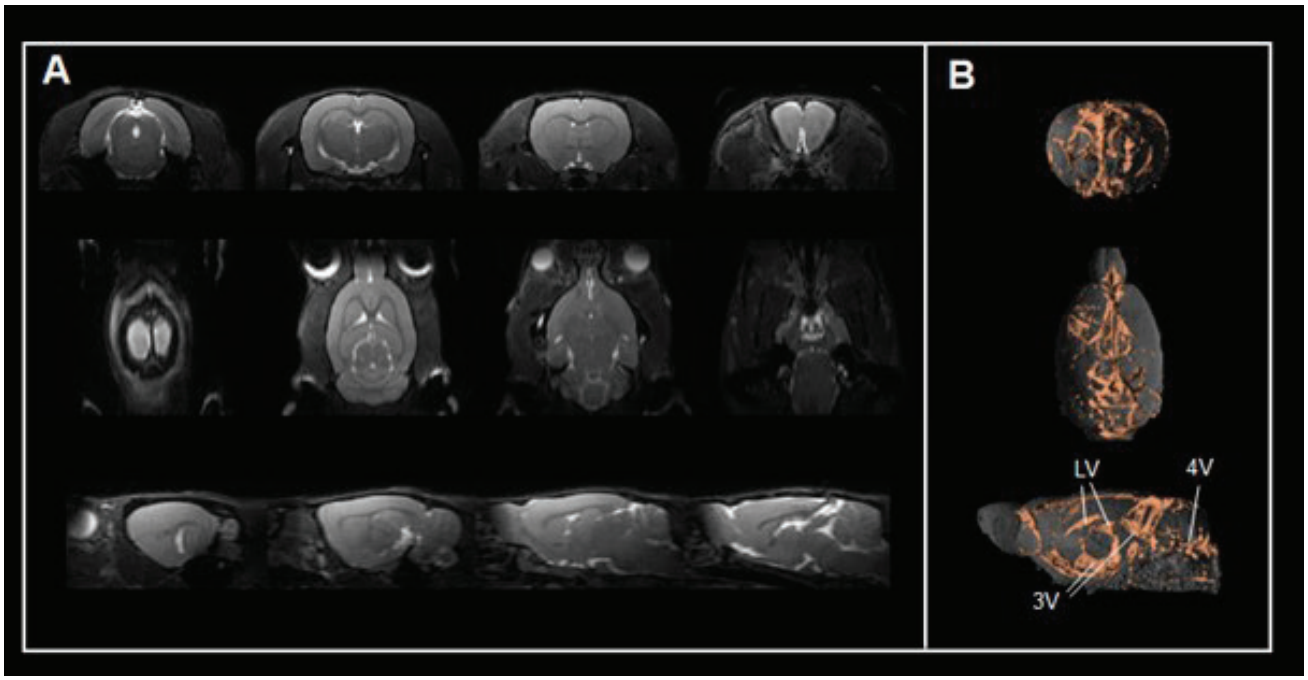

Figure 5. Assessing brain ventricles. A) Axial, coronal, and sagittal T2-weighted TrueFISP images and **B)** corresponding renderings reveal the cerebrospinal fluid spaces in the rat brain. Rat brain data was acquired with a BioSpec Maxwell 94/17 using a volume coil for transmission and a phased-array coil for reception. Tissues were segmented and rendered using PMOD. LV = lateral ventricle; 3V = third ventricle; 4V = forth ventricle

Probing vascular dysfunction

The central nervous system critically depends on the continuous supply of oxygen and nutrients for proper function. A failure in the supply of substrates will result in disturbed homeostasis, tissue damage, and loss of function. Vascular dysfunction (e.g. occlusion, hemorrhage, calcifications) causes neurological deficits in cerebrovascular diseases as well as in neurodegenerative diseases, where vascular alterations can be observed in causal pathways. There are various preclinical MRI techniques to probe the functional properties of the vasculature.[2] Quantitative information about cerebral blood flow (CBF) and cerebral blood volume can be obtained with arterial spin labeling, dynamic susceptibility contrast MRI, vascular space occupancy and other MR methods. An example of a regional CBF (rCBF) map of a mouse brain obtained from a pulsed arterial spin labeling technique is shown in **Fig. 6A**. The map reveals differences in rCBF in different anatomical regions of the brain (cortex = 125.7 ± 36.4 ml/100g/min, thalamus = 204.1 ± 80.5 ml/100g/min). Combining perfusion techniques with pharmacological vasodilatory stimulus allows to assess vascular reactivity of vessels. In addition, dynamic contrast-enhanced MRI can be used to assess the changes in the integrity of the blood-brain barrier, where an impairment

is associated with an influx of intravascular compounds from the blood to the brain. Vascular MRI techniques are employed to assess effects of aging, diet, and environmental factors on vascular function and how this translates into cognitive impairment and tissue damage. Vascular dysfunction can be studied in appropriate disease models as well as in the evaluation of treatments of vascular conditions. Moreover, MRI can provide information about the cardiovascular system (e.g. cardiac function, aortic stiffness) and thus allows investigation of how vascular risk factors affect central nervous system function.

Mapping dysfunction of functional networks and neuronal plasticity

Functional magnetic resonance imaging (fMRI) is used to map changes in neuronal activity in the brain and spinal cord. In fMRI temporal and spatial alterations in the blood oxygen level dependent (BOLD) signal are recorded, commonly with a T2*-weighted gradient echo planar imaging (EPI) sequence. An example of EPI images of the mouse brain acquired with a BioSpec Maxwell 94/17 is shown in **Fig. 6B**. In a task-based fMRI experiment, BOLD signals are measured in response to a stimulus (sensory, thermal, acoustic, pain or other) to identify regions that are functionally involved in a specific task performance. In resting state functional MRI, BOLD data is collected in task-free conditions. The measured low-frequency oscillations allow to evaluate regional interactions, used to outline specific functional networks in the brain and spinal cord and/or to generate a functional connectome that facilitates a global assessment of circuitry and circuit function. During measurements animals are often anesthetized, though fMRI in awake but immobilized mice and rats is increasingly performed, which reduces the confounds of anesthesia and allows to study animals during behavioral tasks. For pixelwise analysis of signal changes, the stability of scanner performance is imperative. An excellent time course stability of repeated EPI acquisitions could be demonstrated with a BioSpec Maxwell 94/17 **(Fig. 6C)**. Drift compensations are applied via additional navigator signals that are acquired and used to apply magnetic field corrections to the working frequency, achieving a temporal signal-to-noise ratio = 123).

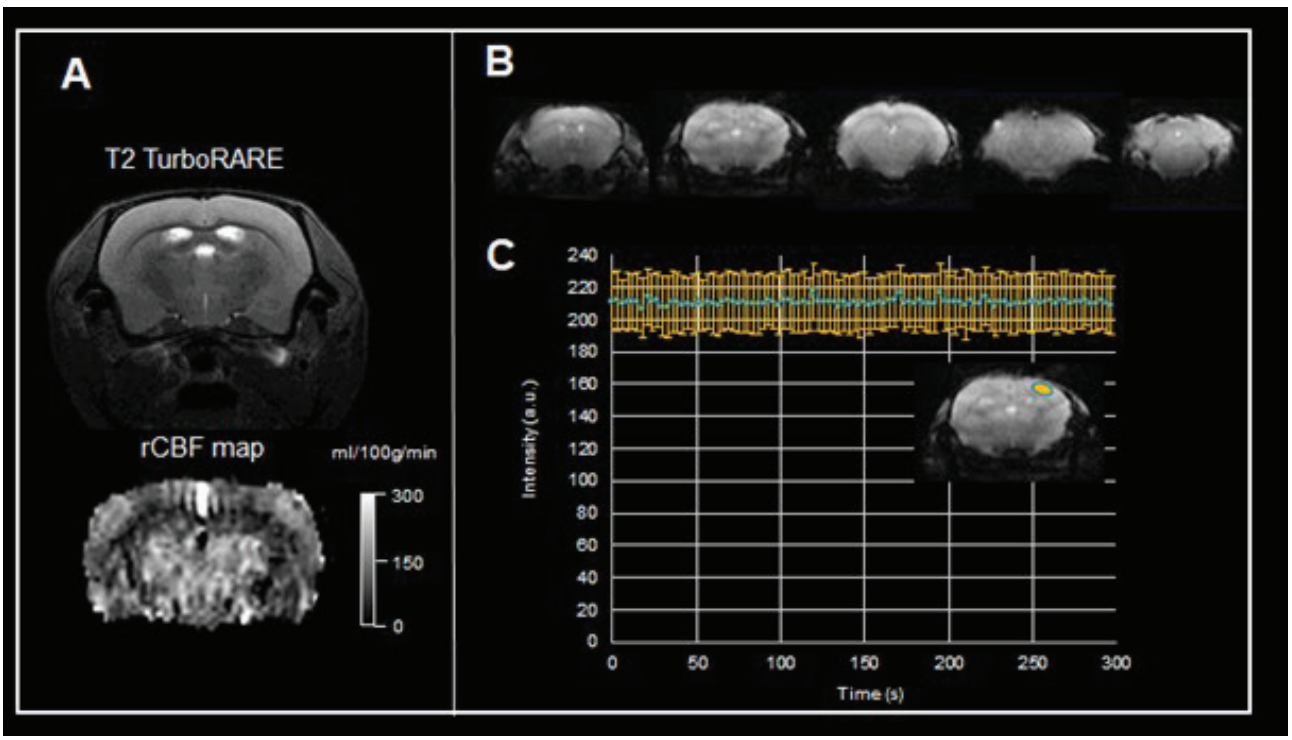

Figure 6. Functional MRI of the brain. A) Pulsed arterial spin labeling of the mouse brain. Shown are a T2-weighted TurboRARE image for anatomical reference, and the rCBF map computed from the flow-sensitive alternating inversion recovery (FAIR)-EPI method. Data were acquired with a BioSpec Maxwell 94/17 using a linearly polarized bird-cage resonator for transmission and a phased-array receive-only surface coil in cross-coil mode under isoflurane anesthesia. **B)** Example of gradient echo EPI images of the mouse brain acquired with a BioSpec Maxwell 94/17 equipped with a 23 mm volume coil. Shown are five 0.6 mm thick axial slices with an in-plane resolution of 141 µm x 156 µm. **C)** The time course characteristics of EPI imaging for fMRI studies were assessed by acquiring 100 repetitions of gradient echo EPI over an acquisition time of 5 minutes with active drift compensation. Acquisition shows solid time course stability with a temporal signal-to-noise ratio = 123.

Functional MRI is used to study dysfunction of the central nervous system on the regional or network level during the development of neurodegenerative diseases and allows to study plasticity and functional recovery of the brain and spinal cord after acute injury. Moreover, resting state functional MRI in combination with behavioral tests allows to construct computational models to relate symptoms to circuit function changes. A limitation of BOLD-fMRI is that it constitutes only an indirect measure of neuronal activity. The BOLD contrast is governed by neurovascular coupling that can itself be altered during development, aging, in diseases conditions and by anesthesia. Thus, an observed change in BOLD signal might not relate to altered neuronal activity if neurovascular coupling is compromised. However, a unique advantage of preclinical fMRI studies is that they can be combined with pharmacological, chemo- and optogenetics or electrical neuromodulation approaches and/or invasive read-outs (electrophysiology, optical imaging, etc.). These approaches can be used to determine the cellular underpinnings of the observed fMRI read-outs, or to perform mechanistic or interventional studies.[3] Moreover, attempts are made to monitor neuronal activity with fMRI directly, thus increasing the spatial and temporal precision.

Conclusion

In summary, preclinical MRI and MRS techniques provide sensitive and specific measures of metabolic and functional processes in the central nervous system that are critically and early involved in the development of neurological diseases. The techniques have become important tools in preclinical neurological research for understanding underlying disease mechanisms, during drug discovery and development, and for providing biomarkers of disease.

List of abbreviations

BOLD = blood oxygen level dependent
CBF = cerebral blood flow
CEST = chemical exchange saturation transfer
CSI = chemical shift imaging
EPI = echo planar imaging
FAIR = flow-sensitive alternating inversion recovery
FLASH = fast low angle shot
fMRI = functional MRI
MRI = magnetic resonance imaging
MRS = magnetic resonance spectroscopy
PET = positron emission tomography
PRESS = point resolved spectroscopy
RARE = rapid acquisition with relaxation enhancement
rCBF = regional cerebral blood flow
TrueFISP = true fast imaging with steady state precession

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All Bruker in vivo animal work was approved by the institutional animal care and use committee (IACUC) or local authorities and conducted under valid study permit.

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