MR Microscopy and DTI of Organotypic Hippocampal Slice Cultures

Katharina Göbel[1], Jochen Leupold[1], Bibek Dhital[1,2], Pierre LeVan[1], Marco Reisert[1], Johannes Gerlach[3], Robert Kamberger[4], Carola Haas[3], Jürgen Hennig[1], Dominik von Elverfeldt[1], and Jan G. Korvink[4]

[1]Medical Physics, Dept. of Radiology, University Medical Center Freiburg, Freiburg, Germany, [2]German Cancer Consortium (DKTK), Heidelberg, Germany, [3]Dept. of Neurosurgery, Experimental Epilepsy Research, University Medical Center Freiburg, Freiburg, Germany, [4]Dept. of Microsystems Engineering (IMTEK), Technical Faculty, University of Freiburg, Freiburg, Germany

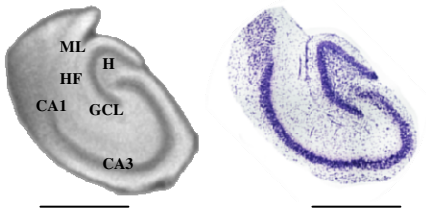
Target audience / Purpose

Organotypic hippocampal slice cultures (OHSC) are a well established neuronal culture system that combines the advantages of cell culturing with a neuronal network tightly reflecting the *in vivo* state. They are frequently used to study morphological, molecular and electrophysiological changes associated with epilepsy[1]. Our aim is to investigate these changes during epileptogenesis in OHSC, particularly using high spatial resolution MR microscopy, whose non-invasiveness would allow continuous longitudinal monitoring near the cellular level[2]. Here we demonstrate high-resolution structural imaging of OHSC, particularly using diffusion tensor imaging (DTI) to finely resolve hippocampal cytoarchitecture[3].

Methods

A commercially available mousehead two-element quadrature cryogenic coil system with a 7 Tesla Bruker BioSpec small horizontal animal scanner (bore size = 20 cm, maximum gradient amplitude = 676 mT/m) was used to adapt MR pulse sequences with respect to OHSC imaging. The standard setup, which is designed for imaging living mice, has been modified by the addition of a 3D printed sample holder made from PLA. Fixed mouse brain slices (hippocampus, 400 µm thick) are then inserted into a PMMA container (height = 3 mm) filled with saline solution and sealed on both sides with adhesive PCR tape to avoid evaporation.

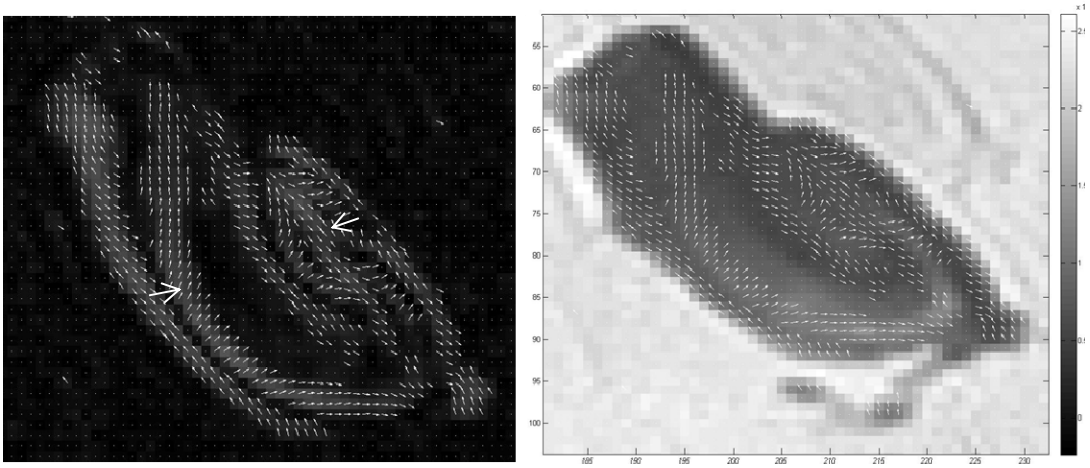
Multi-slice 2D gradient echo sequences were applied with: TR = 300 ms, TE= 7.7 ms, flip angle = 50°, resolution 16×18×150 µm³ obtained in 2h 8min (**A**). The neuronal cytoarchitecture was subsequently compared to optical microscopy of histologically stained tissue sections (see **B**). An adapted spin-echo EPI DTI sequence was used for 2D DTI measurements with TR = 3000 ms, TE= 38.81 ms, resolution 39×39×100 µm³, NEX = 16, 10 repetitions, scan time = 9h 36min. Since we are mainly interested in the planar structural composition, 12 in plane diffusion directions, equally distributed on a semi circle, were chosen.



***A** 2D FLASH, high-resolution image of 16×18×150 µm³ depicting the hippocampal substructure; GCL – Granule cell layer, ML – Molecular layer, H - Hilus, HF – Hippocampal fissure, CA – Cornu ammonis, DG – Dentate gyrus; **B** Cresyl violet staining of 20 µm thick OHSC cryosection.*

Results / Discussion

A comparison of the obtained MR images with histological stainings (**A, B**) shows that a resolution enabling the identification of hippocampal substructures with feature sizes of below 70 µm could be reached. The laminar structure of hippocampal subfields/thin neuronal layers are clearly visible. Fig. **C** shows the reconstructed DTI data of a fixed coronal hippocampus slice illustrated in a fractional anisotropy (FA) map with associated dominant diffusion directions of water molecules expected to correspond to fiber pathways and radially oriented pyramidal and granular cell dendrites (indicated by bold arrows). Fig. **D**, an apparent diffusion coefficient (ADC) map from the same slice as in fig. **C**, also reveals high intensity contrasts in dense neuronal layers. This approach of 2D DTI promises amongst others a gain in signal-to-noise ratio (SNR) and accordingly shorter scan times compared to conventional 3D DTI.



***C** FA map (FA > 0.1), the two bold arrows are highlighting hippocampal structures expected to be the radially oriented pyramidal and granular cell dendrites; **D** ADC map. The arrows show the principle axis of the diffusion tensor.*

Conclusion

The results presented here demonstrate the ability of MR microscopy and DTI to delineate hippocampal subfields with contrast and resolutions that may eventually approach histology. They can set the framework for an *in vitro* system, which serves as a basis for the performance of a broad spectrum of measurements, such as MR spectroscopy and fiber tracking, providing deeper insights into the dynamic processes of epileptogenesis. A fundamental understanding of these processes is a necessity to overcome the technological challenges associated with *in vivo* studies as well as an early diagnosis and the specific treatment of epilepsy.

References: [1] S. Tinnes et al., TIMP-1 inhibits the proteolytic processing of Reelin in experimental epilepsy, FASEB Journal, 2013;27(7):2542-52. [2] J.J. Flint et al., Magnetic resonance microscopy of human and porcine neurons and cellular processes, NeuroImage 2012;60:1404-1411. [3] L. Harsan et al., In vivo diffusion tensor magnetic resonance imaging and fiber tracking of the mouse brain, NMR in Biomed., 2010;23:884-896.

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