Micro-MR correlates of cellular-level alterations in epileptogenesis

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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, chemical and connectivity changes associated with epilepsy[1]. Our aim is to investigate these changes during epileptogenesis using high spatial resolution MR microscopy, whose non-invasiveness allows continuous longitudinal monitoring near the cellular level[2]. Here we demonstrate high-resolution structural imaging of OHSC to resolve hippocampal cytoarchitecture[3,4] and connectivity patterns of epileptic and healthy tissue.

Methods

A commercially available mousehead two-element quadrature cryogenic coil system with a 7 Tesla Bruker BioSpec 70/20 small animal scanner (bore size = 20 cm, maximum gradient amplitude = 676 mT/m) was used to adapt MR pulse sequences with respect to OHSC imaging. Up to now, five fixed slices (400 µm thick) of kainate injected and control animals were 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= 13 ms, flip angle = 50°, resolution 18×18×104 µm³ obtained in 2h 56min. An adapted spin-echo EPI DTI sequence was used for DTI measurements with TR = 3000 ms, TE= 39 ms, resolution 39×39×100 µm³, NEX = 16, 60 directions, scan time = 3h 31min. The neuronal cytoarchitecture was subsequently compared to optical microscopy of histologically stained tissue sections.

Results / Discussion

The morphological MR images reveal strong differences between the epileptic and the healthy control slices: While in the control slice the densely packed neuronal cell layers (cornu ammonis: CA, granule cell layer: GCL) can easily be identified, the GCL of the epileptic animal is strongly dispersed (Fig. 1, top). The strongest and most consistent DTI signals can be found in areas of dendritic trees of granule cells and pyramidal cells in CA1 (white arrows) as well as in mossy fibers and axonal processes in the hilus, alveus and Schaffer collaterals (asterisks) (Fig. 1, bottom). The connectivity pattern within the slice of the epileptic animal is strongly changed in comparison to the control slice. The dendritic tree signals of CA1 pyramidal cells seem to be almost completely lost, which is confirmed by the MRI and immunohistochemical data, as CA1 cells are widely lost (Fig. 1, 2nd row). The Golgi stainings highlight these morphological changes occurring during epileptogenesis which most likely can be considered as the anatomical basis of the MRI and DTI signal changes. While in the control slice compact cell layers containing granule cells (GCL) and pyramidal cells (CA1 & CA3) with radially oriented dendritic trees are visible (white arrows), only dendrites of granule cells can be found in the epileptic slice (Fig. 1, 3rd row).

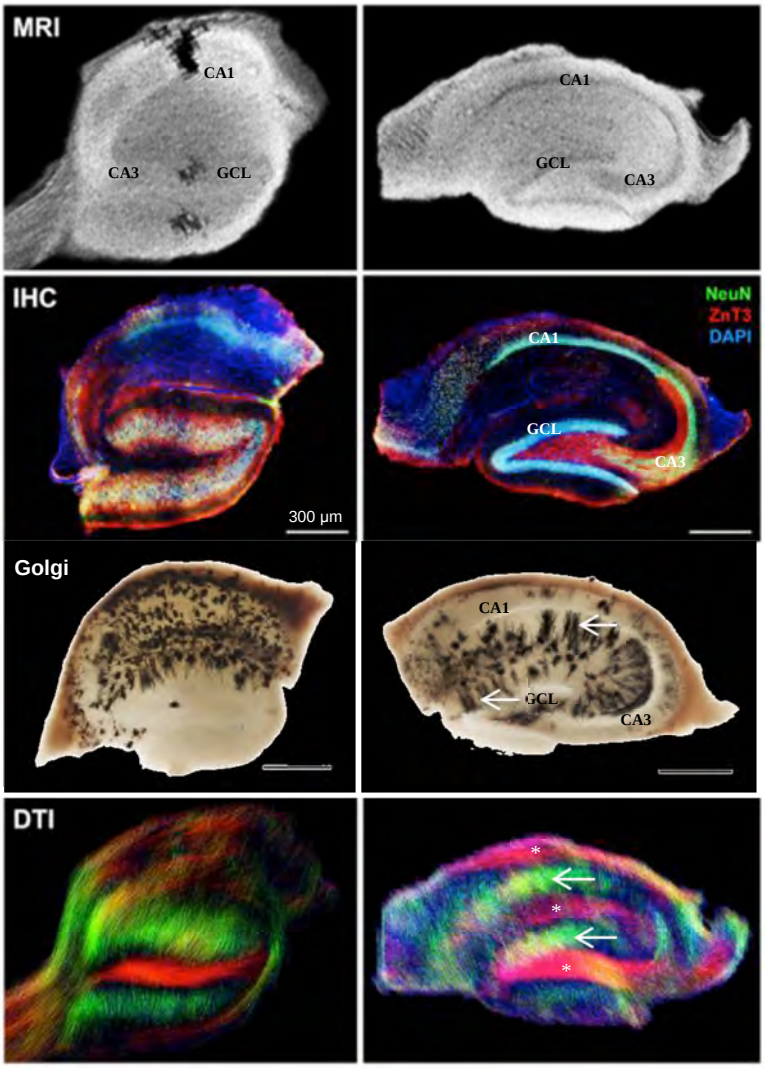

Figure 1 *Magnetic resonance microscopy (MRI), immunohistochemical (IHC) and Golgi staining as well as diffusion tensor imaging (DTI) images of representative hippocampal brain slices from kainate-treated (left) and control mice (right).*

Conclusion

This work demonstrates that micro-MR can provide deeper insights into the dynamic processes of epileptogenesis at the cellular level in *in vitro* preparations. A fundamental understanding of these processes is a necessity to overcome the technological challenges associated with *in vivo* studies as well as to find new markers for 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. [4] Göbel K. et al., MR Microscopy and DTI of Organotypic Hippocampal Slice Cultures. In: Proc ISMRM, Milan, Italy, 2014, p. 1517.

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