MR MICROSCOPY STUDIES OF RAT HIPPOCAMPAL SLICE CULTURES

T.M. Shepherd, B. Scheffler, M.A. King (UF Neuroscience); G.J. Stanisz (U Toronto, Canada);
D.A. Steindler, S.J. Blackband (UF, Neuroscience)

Introduction

Brain slices provide a useful nervous tissue model to investigate the relationships between magnetic resonance imaging (MRI) contrast mechanisms and tissue microstructure; yet, these acutely isolated tissues remain viable for only 10– 12 h. To study slower biological processes, this work describes the first MR microscopy characterization of organotypic rat hippocampal slice cultures that can be maintained for several weeks.

Experimental

MRI data were obtained at room temperature using a 10-mm birdcage coil interfaced to a Bruker 14.1-T vertical magnet and console with 3000-mT/m imaging gradients and a novel multislice perfusion chamber. Images for analysis with the two-compartment analytical model of tissue microstructure had low in-plane resolution (128x64 matrix, 1.5 cm FOV) to improve signal-to-noise while reducing the time required per scan. Water diffusion measurements in slice cultures employed a pulsed-gradient spin echo multislice sequence with diffusion times (Td) of 10, 20, and 35 ms. For each diffusion time, 12 different diffusion gradient strengths were employed so that each Td measurement yielded 12 diffusion weighted images with b values between 7 and 10,000 s/mm2 (including imaging cross-terms). Additional higher resolution diffusion-weighted and gradient echo images were collected from several cultured hippocampal slices to assess the laminar anatomy of the hippocampal slice cultures. Diffusion-weighted images at 78-Am in-plane resolution were acquired in 3.5 h.

Results and Discussion

Figure 1 shows example images of cultured slices. These cultures tolerated the immersion conditions at 20- or 35-C required for MRI study for 4+ h before significant changes were observed. With phase-contrast optical microscopy, no structural pathology was observed in hippocampal slice cultures at 20- or 35-C even after 8-h immersion. Few pyknotic changes or apoptotic bodies were observable with 4V,6-diamidino-2-phenylindole (DAPI) staining even after 8-h immersion. Glial fibrillary acidic protein (GFAP) immunoreactivity in rat hippocampal slice cultures increased significantly from background levels only after 4 h immersion at 20-C. No regional differences within the slice cultures for responses to increasing immersion time were observed.

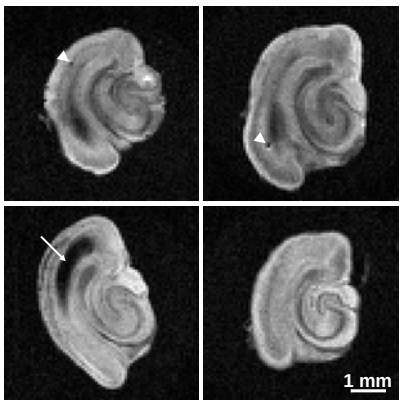

Figure 1. Example images of cultured rat hippocampal brain slices.

Conclusions

This study demonstrates the feasibility of MRI investigations using cultured rat hippocampal slice cultures incubated for 2+ weeks prior to MRI characterization. Immunohistochemistry indicated that slice cultures tolerated the conditions required for MRI study well. MRI of cultured tissue slices is highly amenable to correlative microscopy techniques and offers great promise for future MRI investigations of pathological tissue reorganization, molecular imaging and stem cell therapies. These studies have now been published (1).

References.

(1) Shepherd TM et al., Neuroimage. 2006 Apr 15;30(3):780-6.

Acknowledgements

Support was provided by NIH grants R01-NS36992, P41 RR 16105, R21 NS46384, NS37556, and the NHMFL.