MR Microscopy of Rat Hippocampal Slice Cultures: a Novel Model for Studying Cellular Processes and Chronic Perturbations to Tissue Microstructure

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INTRODUCTION

Many current questions in magnetic resonance imaging research, such as understanding the biophysical basis for water diffusion in nervous tissue [1], may require experiments in high-field magnets with powerful imaging gradients and long acquisition times. Experiments that meet these criteria require alternative tissue models because the experiments are presently difficult, if not, impossible to conduct using animal or human subjects. Novel technological developments, such as MRI-based molecular imaging and stem cell imaging, also may benefit from simplified tissue models imaged at high fields that provide near-complete experimental control over the tissue environment. Brain slices provide a useful nervous tissue model for these scientific questions, but only remain viable for 10-12 hrs and are too thick for correlative techniques like confocal microscopy. To study slower biological processes, this work describes the first MRI characterization of 150-μm thick rat hippocampal slice cultures, which can survive 30+ days and tolerate MRI studies at temperatures of 35°C.

METHODS

Rat hippocampal slices were cultured as described previously [2]. After 2-weeks incubation, individual slice cultures were carefully cut from the culture membranes under sterile conditions such that the outer edges of the remaining membrane with attached slice culture approximated 9-mm in greatest dimension. The outer edges of the cut slice culture membrane then were compressed between two 200-μm thick Delrin rings as it was gently immersed in culture media inside a 10-mm NMR tube [3]. This step can be repeated such that as many as 20-30 slice cultures can be imaged simultaneously. Diffusion, T_1 and T_2 measurements suitable for a two-compartment analytical model of water diffusion [4] were collected from 11 rat hippocampal slice cultures at 20 and 37°C using a 10-mm Helmholtz pair coil interfaced to a Bruker 14.1-T vertical magnet and console with 3000 mT/m imaging gradients. To assess the laminar anatomy of the hippocampal slice cultures, additional 78-μm in-plane resolution diffusion-weighted (thickness = 100 μm, TR/TE = 1500/23.3 ms, 64 averages, T_d = 10 ms, b = 2028 s/mm^2) and 39-μm in-plane resolution T_2*-weighted gradient echo images (thickness = 80 μm, TR/TE = 185/12.5 ms, flip angle = 30°) of rat hippocampal slice cultures were acquired. Additional slices were treated under conditions that mimicked the MRI data acquisition and processed with immunohistochemistry for glial fibrillary acidic protein (GFAP), DAPI and neuron-specific nuclear protein (NeuN) using standard protocols.

RESULTS

The methods described here were simple and provided high quality MRI datasets from rat hippocampal slice cultures. The laminar anatomy of the hippocampus, entorhinal and temporal cortex were well-preserved in rat hippocampal slices that had been cultured for 2+ weeks (Fig. 1). MRI of the rat hippocampal slice cultures in a 14.1-T magnet with a 10-mm Helmholtz-pair coil provided excellent SNR per unit time (e.g. an SNR of 14.7:1 at b = 10000 s/mm^2 with 2.74 nL voxels acquired in 6 minutes). Diffusion-weighted signal attenuation curves in the rat hippocampal slice cultures were non-monoexponential at all diffusion times studied. The two-compartment model with exchange [4] fitted the diffusion MRI data well and provided estimates of the extracellular apparent diffusion coefficient (0.694 ± 0.066 μm^2/ms), apparent restriction diameter (3.35 ± 0.50 μm), water exchange rate (69.3 ± 14.1 s^{-1}) and intracellular water fraction (0.372 ± 0.038, no units). The T_1 and T_2 values of slice cultures were determined to be 1.936 ± 0.048 s and 0.064 ± 0.005 s respectively [mean ± SD, 11 slices]. Slice cultures tolerated the immersion conditions at 20° or 35°C required for MRI study well for more than 4 hours before significant changes were observed with phase-contrast microscopy, GFAP immunoreactivity or NeuN staining.

DISCUSSION

This study demonstrated the feasibility of MRI investigations using cultured rat hippocampal slice cultures incubated for 2+ weeks. Despite the inherent thinness of slice cultures (~ 150 μm), diffusion MRI of slice cultures using even heavy diffusion-weighting (b ~ 10,000 s/mm^2) had reasonable SNR (> 12:1) in acquisition times less than 10 min. These images had sufficient resolution to distinguish the different cytoarchitectural regions of the cultured rat hippocampal slices. Cultured rat hippocampal slices exhibited comparable MRI-observed tissue microstructure to *in vivo* rat nervous tissue and rat/human brain slices [5]. Cultures also maintained excellent health under the conditions required for MRI investigation for more than 4 hours at 20° or 35°C. Cultured rat brain slices can survive for 30+ days after procurement and be re-cultured after initial MRI characterizations. Also, without the strict necessity of perfusion, this setup can be simpler than methods for MRI of acutely-prepared brain slices [5]. Although the thinness of slice cultures creates additional challenges to obtaining sufficient SNR, this thinness is more amenable to correlative techniques (e.g. confocal microscopy) that may prove essential to certain MRI investigations of tissue microstructure. Slice cultures may enable MRI investigations of stem cell transplantation and migration, or MRI characterizations of chronic perturbations to tissue microstructure (e.g. viral-induced formation of Alzhemier's-like plaques). Cultured brain slices also may provide an experimental platform for the development of molecular imaging technologies.

REFERENCES

1. Norris DG. NMR Biomed 14:77-93 (2001) 2. Scheffler et al. 130:5533-5541 (2003). 3. Shepherd et al. Neuroimage *in press* (2006). 4. Li et al. MRM 40:79-88 (1998). 5. Shepherd et al. MRM 49:856-863 (2003). Funded by NIH RO1 NS36992 & P41 RR16105. Thanks to Dan Plant for technical assistance.

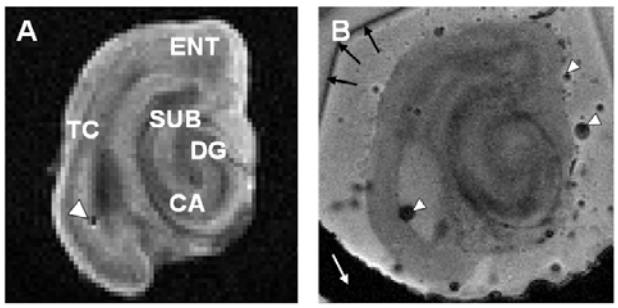

Figure 1 - Diffusion weighted MRI (A) and gradient-echo MRI (B) demonstrate the laminar anatomy of a rat hippocampal slice culture well (TC = temporal cortex, ENT = entorhinal cortex, SUB = subiculum, DG = dentate gyrus, CA = cornu ammonis). Free edges of the slice culture membrane (black arrows), the Delrin compression ring (white arrow) and air bubbles (arrowheads) are seen in panel B.