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NeuroImage 30 (2006) 780 – 786

MR microscopy of rat hippocampal slice cultures: A novel model for studying cellular processes and chronic perturbations to tissue microstructure

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Received 16 June 2005; revised 23 September 2005; accepted 14 October 2005
Available online 1 December 2005

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 MRI microscopy characterization of organotypic rat hippocampal slice cultures that can be maintained for several weeks. Diffusion-weighted images of slice cultures acquired with a 14.1-T magnet demonstrated the laminar anatomy of the hippocampus with relatively high signal-to-noise ratios. Diffusion data analyzed using a two-compartment model with exchange indicated that cultured slices had a comparable microstructure to acute brain slices and to in vivo brain. 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.

Keywords: Heterotypic; Stem cell; Molecular imaging; Brain slice

Introduction

Many current questions in magnetic resonance imaging (MRI) research, such as understanding the biophysical basis for water diffusion in nervous tissue (Norris, 2001), cannot be addressed adequately in animal or human subjects due to hardware limitations and limitations imposed by the nature of the sample. One solution to these challenges is to conduct MRI microscopy experiments on viable, perfused brain slices prepared from animals or human surgical biopsy specimens using high-field MRI systems that incorporate strong gradients (Shepherd et al., 2003a). These brain slices retain the 3-dimensional cytoarchitecture of in vivo nervous tissue and tolerate relatively long imaging protocols in high-field, narrow-bore magnets. In addition, such brain slices no longer have a blood–brain barrier, which gives complete experimental control over the extracellular environment of the tissue. These properties have enabled studies of the water diffusion changes that follow pharmacological or osmotic perturbations to rat and human hippocampal slices (Blackband et al., 1995; Bui et al., 1999; Shepherd et al., 2003a). Recently, this experimental method also facilitated the measurement of mean intracellular residence time of water in rat and human cortical slices (Shepherd et al., 2004), which has proven difficult to measure accurately in vivo. These types of studies may provide insight into the fundamental origins of MR signals in tissues and how these signals may be interpreted in terms of detecting and monitoring the treatment of disease states such as brain ischemia.

However, brain slices that are obtained for immediate experiments from euthanized animals or human surgical biopsy specimens have some limitations that prevent their more widespread use as a tissue substrate for MRI experiments and technology development. These "acutely prepared" brain slices appear viable by electrophysiology, histology, and diffusion MRI for only 10–12 h when imaged at room temperature (20°C) with periodic perfusion (Shepherd et al., 2003a,b; Shepherd et al., 2002). This relative hypothermia is neuroprotective and facilitates longer experiment times but may also blunt normal and pathological responses of the nervous tissue to experimental perturbation. Further, proteolytic enzyme cascades may be activated by the slice procurement

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.neuroimage.2005.10.020

process despite normal-appearing cell and tissue morphology in the slices. The slices also have 50-μm deep zones of surface tissue injured during the procurement process (Aitken et al., 1995). These zones must be excluded from MRI analysis and necessitate that acutely prepared slices are several hundred microns thick, which in turn makes it difficult to use correlative techniques, like confocal microscopy, on live slices.

In this report, we describe a novel method that provides high-quality MRI data from cultured rat hippocampal slices imaged using a 14.1-T magnet. Slice cultures share the advantages of acutely prepared brain slices but remain viable and organized organotypically for several weeks. Cultured slices also do not require perfusion for some imaging studies even at 35°C. Slice cultures may enable future MRI investigations of stem cell transplantation and migration (Scheffler et al., 2003; Benninger et al., 2003) or MRI characterizations of chronic perturbations to tissue microstructure (e.g., Alzheimer's plaques) (Harrigan et al., 1995; Brendza et al., 2003). The stable and controllable biochemistry of cultured brain slices also suggests that this method may provide an excellent experimental tissue platform for the development of molecular imaging technologies (e.g., MRI of gene expression) (Louie et al., 2000).

Methods

Culture of rat hippocampal slices

The use of rodents for this study was approved by the University of Florida Institutional Animal Care and Use Committee. The methods used to culture hippocampal slices for this study were described previously (Scheffler et al., 2003; Benninger et al., 2003). Briefly, 375-μm slices were cut horizontally from the brain of P9 Wistar rats (Charles River, Wilmington, MA) using a vibratome (VS1000, Leica, Bannockburn, IL). Slices were placed on polyethylene membrane inserts (Costar, Corning, NY, USA) and cultured under interphase conditions in a humidified 5% CO_2 atmosphere at 35°C (Stoppini et al., 1991). For the first 5 days, slices were cultured in horse serum-containing medium increasingly replaced by serum-free, defined solution based on DMEM-FI2 with N2 and B27 supplements. Functional and morphological tissue integrity for up to 5 weeks in culture were reported previously (Scheffler et al., 2003; Benninger et al., 2003).

Preparation of slice cultures for MRI

After a 2-week incubation, individual rat hippocampal slice cultures were carefully cut from the culture membranes with a scalpel and fine surgical scissors such that the outer edges of the remaining membrane with attached slice culture approximated the inner diameter of a 10-mm NMR tube (Fig. 1). A bottom Delrin anchor with attached hollow tubing was then immersed into a 10-mm NMR tube filled with fresh slice culture media.

Two 200-μm-thick Delrin rings were designed to compress the outer edges of the cut slice culture membrane (Fig. 1). Both Delrin rings had a 9-mm outer diameter to fit inside a 10-mm NMR tube and a notch cut to allow passage of the tubing attached to the bottom Delrin anchor. The bottom ring had a narrow inner diameter (4 mm) to provide additional support for the slice culture membrane that rests on top of it while the top ring had an 8-mm inner diameter to prevent compression of the

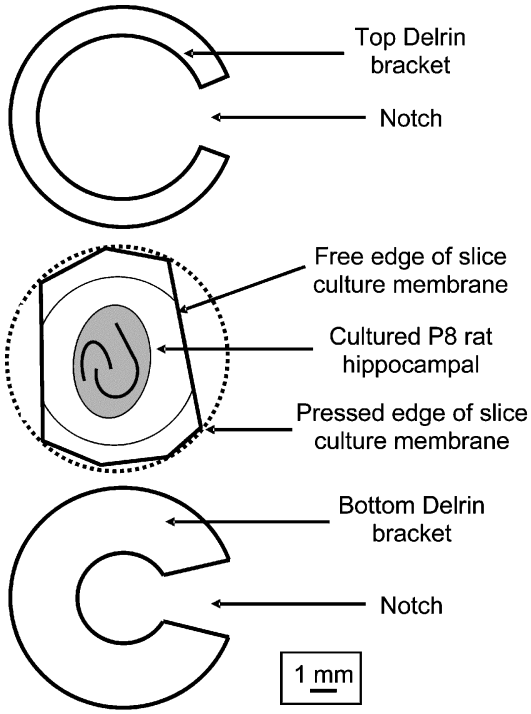

Fig. 1. Schematic of assembly used to obtain MRI of rat hippocampal slice cultures. The slice culture and attached membrane are cut away from the cell culture inserts and trimmed to fit inside a 10-mm NMR tube (inner diameter ~9 mm). The edges of the culture membrane then are pressed between the top and bottom Delrin brackets. Notches in the Delrin brackets are used for passage of a Delrin anchor, which can then be used to remove the sample after imaging is complete.

actual nervous tissue culture. Working just below the meniscus of the culture media in the NMR tube, the bottom ring was placed into the NMR tube, followed by the culture membrane with attached slice culture, then the thinner top ring was added to press the culture membrane flat and horizontal. It was critical that the tissue-covered surface of the slice culture membrane point upwards to avoid compressing the cultured slice against the thicker bottom ring. This step can be repeated such that as many as 20–30 slice cultures can be imaged simultaneously—in this report, only 5 slice cultures were stacked at a single time. A top Delrin piece then was placed over the assembly to protect the top slice cultures from contact and to maintain consistent compression on the Delrin rings. The resultant side profile of this stacked setup for slice cultures would appear similar to the schematic shown in a previous work for acutely prepared brain slices (Shepherd et al., 2002).

The resultant assembly was slowly pressed to the bottom of a 10-mm NMR tube with a glass rod. Additional outflow lines can be added to withdraw perfusate if continuous perfusion of the slice cultures were required during the MRI experiment (see Shepherd et al., 2002). The culture media above the slice cultures also can be gassed with 95% O_2/5% CO_2 to prolong slice viability by maintaining high oxygen tensions and buffering physiological pH (7.4) in the media. The tube was tightly sealed with a slotted NMR-tube cap and parafilm. This assembly process must occur under sterile conditions to avoid infection if live slice cultures are to be used for experiments longer than 10–12 h (e.g., time-intensive MRI studies, subsequent correlative

microscopy, or re-incubation of cultures). Autoclave sterilization may distort the fine tolerances of the Delrin pieces so it is preferable to treat the equipment with ethylene oxide gas for sterilization when desired.

MRI of slice cultures

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. Pilot multislice axial, sagittal and coronal T_1-weighted images were used to locate the positions of the Delrin rings. Pilot diffusion-weighted images helped locate the cultured nervous tissue slices due to their significantly restricted water diffusion compared to culture media. The diffusion-weighted pilot scans then were used to optimize the positions of 100-μm thick axial MR-defined slices through the center of the approximately 150-μm thick rat hippocampal slice cultures. Diffusion, T_1 and T_2 measurements suitable for a two-compartment analytical model of water diffusion (Stanisz et al., 1998) were collected from 11 rat hippocampal slice cultures. Additional slice cultures that showed substantial volume averaging with ACSF perfusate ($N = 2$) or large susceptibility artifacts from air bubbles ($N = 2$) were rejected from subsequent imaging studies and analysis.

Images for analysis with the two-compartment analytical model of tissue microstructure had low in-plane resolution (128 × 64 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 (T_d) of 10, 20, and 35 ms. The gradient duration (δ) was 3 ms. Previous studies of hippocampal slices demonstrated that diffusion gradient orientation has minimal impact on the water diffusion observed at the image resolutions used here (Blackband et al., 1995), so only slice-oriented diffusion gradients were used in these experiments to impart diffusion weighting. For each diffusion time, 12 different diffusion gradient strengths were employed so that each T_d measurement yielded 12 diffusion-weighted images with b values between 7 and 10,000 s/mm^2 (including imaging cross-terms). Each diffusion-weighted image had 4 averages with a 1.5-s repetition time, while echo time was minimized with respect to T_d (23.3, 33.3 and 48.3 ms respectively). Thus, the acquisition took 6 min 24 s per diffusion-weighted image or 1.25 h per T_d acquisition. T_1 values were determined in 20 min with a partial saturation experiment using 8 different repetition times between 125 ms and 10 s (TE = 10 ms). T_2 values were determined in 11 min with a multiecho sequence using 30 consecutive 10-ms echo images (TR = 10 s). Signal-to-noise ratios (SNR) were calculated based on the mean slice culture signal minus the mean noise divided by the standard deviation of the noise. The two-pool diffusion model with exchange (Stanisz et al., 1998) was fitted to the T_1, T_2, and diffusion data.

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-μm in-plane resolution were acquired in 3.5 h (thickness = 100 μm, TR/TE = 1500/23.3 ms, 64 averages, T_d = 10 ms, b = 2028 s/mm^2). T_2*-weighted gradient echo images of rat hippocampal slice cultures at 39-μm in-plane resolution were acquired in 25 min (thickness = 80 μm, TR/TE = 185/12.5 ms, flip angle = 30°).

Assessment of slice culture viability

To simulate the conditions experienced by slice cultures during the MRI experiments, additional slices were cut from their membrane inserts and immersed in culture media inside 6-well dishes. The volume of media per well was similar to the total volume of media per slice in the 10-mm NMR tubes during the MRI experiments. Slices were incubated either in sealed dishes at room temperature or in dishes inside a culture incubator containing 5% CO_2 at 35°C. For each group, 3 slice cultures were incubated for either 2, 4, 8, or 12 h prior to immersion–fixation with 4% formaldehyde in phosphate buffer (pH 7.4). Slice cultures were visualized with phase-contrast microscopy immediately prior to fixation to assess the structural integrity of the different regions of the hippocampus and entorhinal cortex. After chemical fixation, 25-μm thick slices sections were prepared on a cryostat and processed with immunohistochemistry for glial fibrillary acidic protein (GFAP; 1:400, DAKO, Carpinteria, CA), or neuron-specific nuclear protein (NeuN; 1:50, Chemicon, Temecula, CA), and nuclei were labeled with 0.8 μg/ml 4′,6-diamidino-2-phenylindole (DAPI, Sigma) using standard published protocols (Scheffler et al., 2003). GFAP immunoreactivity was used to assess astroglial reactions, DAPI staining was used to assess changes in nuclear morphology, and NeuN staining was used to assess morphological changes in neuronal somata associated with the immersion conditions required for MRI study of slice cultures.

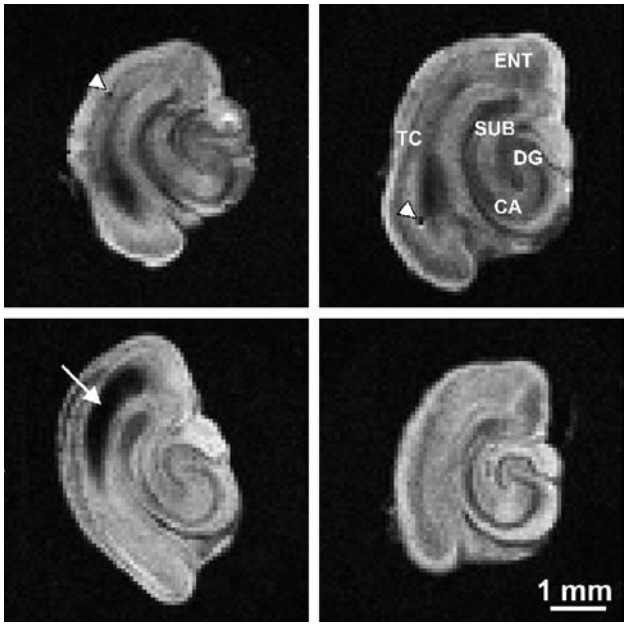

Fig. 2. 78-μm in-plane resolution diffusion-weighted images (b = 2028 s/mm^2) of 4 axially cut, P9 rat hippocampal slices after incubation for 2 weeks [100-μm slice thickness, TR/TE = 1500/23.3 ms, 64 averages, scan time = 3.5 h]. Despite the limited thickness of these hippocampal slice cultures (~150 μm), there is sufficient MRI signal to clearly demonstrate the laminar anatomy of the hippocampus (CA), dentate gyrus (DG), subiculum (SUB), entorhinal (ENT), and temporal cortex (TC). The region of decreased signal within the slices (arrow) atrophies after slice procurement. Microbubbles noted in the images (arrowhead) were trapped underneath the cell culture membrane inserts during chamber assembly.

Results

The laminar anatomy of the hippocampus, entorhinal, and temporal cortex were well-preserved in rat hippocampal slices cultured for 2+ weeks then prepared for MRI characterization (Fig. 2). A small region of subcortical white matter in the temporal cortex region appears to atrophy during the culture process due to axonal transection during the slice procurement process. The regions of the hippocampus (e.g., CA1, CA3, dentate gyrus, and subiculum) are readily discernible in diffusion or gradient echo MRI of the slice cultures (Figs. 2 and 3). It also was possible to distinguish different cytoarchitectural layers within these regions, such as the molecular layer, granule cells, and hilus of the dentate gyrus. Even with high-resolution image matrices and significant diffusion weighting, MRI of the rat hippocampal slice cultures at 14.1-T with a 10-mm birdcage coil provided excellent SNR per unit time (e.g., 14.7:1 at $b = 10000$ s/mm^2 with 2.74 nL voxels in 6 min) (Table 1). Diffusion-weighted signal attenuation curves in the rat hippocampal slice cultures were non-monoexponential at all diffusion times studied (Fig. 4).

The significant dispersion of the signal attenuation curves at diffusion times of 10, 20, or 35 ms suggested that water diffusion in the slice cultures was affected by restriction and/or exchange between at least two unique diffusing water compartments within the tissue during the timescale of the experiment. A two-compartment model with exchange fitted to the experimental diffusion MRI was statistically valid ($\chi^2 < 2$) and provided estimates of the intracellular diffusion coefficient (1.46 ± 1.51 μm^2/ms), extracellular apparent diffusion coefficient that takes into account the tortuous nature of extracellular diffusion (0.69 ± 0.07 μm^2/ms), the apparent restriction diameter (3.4 ± 0.5 μm), the water exchange rate constant (69 ± 14 s^{-1}), and the intracellular water fraction (intracellular relative spin density) (0.37 ± 0.04, no units) [mean ± SD, 11 slices]. The T_1 and T_2 relaxation times of slice cultures were determined to be 1.94 ± 0.05 s and 64 ± 11 ms respectively [mean ± SD, 11 slices].

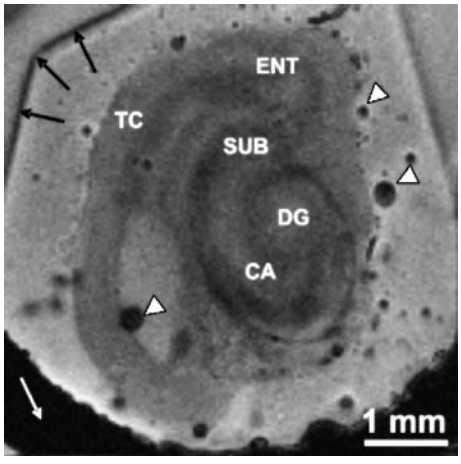

Fig. 3. Gradient echo image of a rat hippocampal slice culture (39-μm in-plane resolution, thickness = 80 μm, TR/TE = 185/12.5 ms, flip angle = 30°, time = 25 min). Gradient echo contrast distinguishes several regions of hippocampal anatomy (TC = temporal cortex, ENT = entorhinal cortex, SUB = subiculum, DG = dentate gyrus, CA = Cornu Ammonis) and could be used to track stem cells or molecular imaging agents labeled with T_1 or T_2 contrast agents. The image includes the free edges of the culture membrane (black arrows) and the top Delrin bracket (white arrow) (see Fig. 1 schematic). Microbubbles of air can form under the surface of the cut culture membrane (e.g., white arrowheads).

Table 1
Signal-to-noise ratios for diffusion-weighted MRI of rat hippocampal slice cultures using 2.74-nL voxels (Acquisition time = 6 min) [mean ± SD, 11 slices]

Diffusion time (ms)	Echo time (ms)	SNR ($b \sim 0$ s/mm^2)	SNR ($b \sim 10{,}000$ s/mm^2)
10	23.3	56.9 ± 4.3	14.7 ± 1.3
20	33.3	50.1 ± 5.9	14.1 ± 1.8
35	48.3	38.0 ± 6.8	12.6 ± 2.3

Slice cultures tolerated the immersion conditions at 20° or 35°C required for MRI study for 4+ h before significant changes were observed (Fig. 5). 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 4′,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. Similarly, neuron-specific nuclear protein (NeuN) staining indicated some reductions in neuronal soma sizes after 4-h immersion at 20°C. No regional differences within the rat hippocampal slice cultures for responses to increasing immersion time were observed (e.g., CA3 versus CA1). The addition of 95% O_2/5% CO_2 to the slice cultures immersed at 35°C largely mitigated the anticipated acceleration in pathological changes such that the GFAP and NeuN changes described above also were not observed until after 4-h immersion at 35°C. Notably, there was significant variability in GFAP immunoreactivity and neuronal size (as indicated by NeuN staining) for the individual rat hippocampal slice cultures within the same treatment groups (e.g., some slice cultures incubated at room temperature for 4 h had different GFAP staining appearances than other slice cultures under the same conditions).

Discussion

This study demonstrates the feasibility of MRI investigations using cultured rat hippocampal slice cultures incubated for 2+ weeks prior to MRI characterization. Despite the inherent thinness

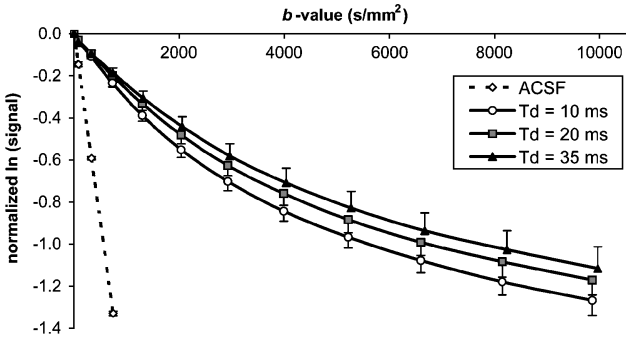

Fig. 4. Normalized log–linear plot of diffusion-weighted signal attenuation curves for rat hippocampal slice cultures at three different diffusion times (Td) [mean ± SD, 11 slices]. Dispersion of the three curves indicates that signal attenuation is affected by restriction and/or exchange at the diffusion times used (10–35 ms).

A

	0 hrs	1 hrs	2 hrs	4 hrs	8 hrs
Structural Damage	-	-	-	-	-
Neuron shrinkage	-	-	+/-	+	++
Reactive Gliosis	+	+	+	++	+++

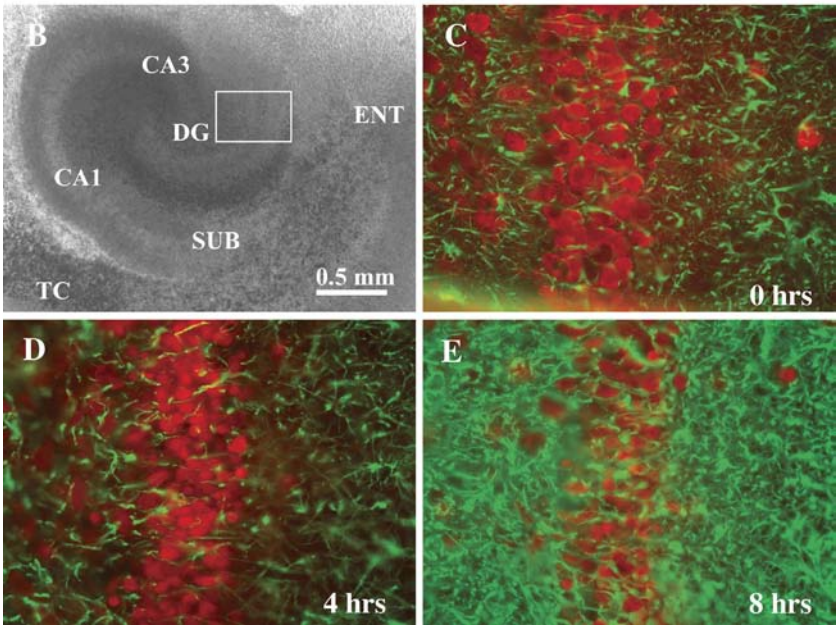

Fig. 5. The viability of slice cultures under the conditions required for MRI studies was qualitatively assessed by immersing slices cultured for 15 days in culture media for up to 8 h at 20°C or 35°C (A). Phase-contrast microscopy of cultured hippocampal slices after 4-h immersion did not indicate structural pathology (B) [white box indicates field-of-view for panels C–E]. Further, NeuN and GFAP fluorescent immunostaining indicate relative stability up to 4 h after immersion (C–D) but demonstrate some neuronal soma shrinkage and increased GFAP immunoreactivity consistent with reactive gliosis after 8-h incubation (E).

of slices at this stage in culture (~150 μm thick), diffusion MRI of slice cultures using even strong diffusion weighting had reasonable SNR in acquisition times less than 10 min. High SNR was also readily available from less signal-attenuated MRI contrast mechanisms, such as T_1, T_2, or gradient echo weighted images. These images provided more than adequate resolution and contrast to distinguish the different cytoarchitectural lamina of the cultured rat hippocampal slices. The T_1 values of cultured slices were indistinguishable from acute brain slices, but mean T_2 values were reduced approximately 35% from 98 ms in acute brain slices (Shepherd et al., 2004) to 64 ms in cultured slices. This may be attributed to increased iron in the culture media used during the MRI acquisition.

Although from different brain regions (hippocampus versus neocortex), it is interesting to compare the mean parameters obtained from the two compartment model with exchange (Stanisz et al., 1998) for the cultured hippocampal slices with previous model data from acute cortical brain slices (Shepherd et al., 2004). The mean fraction of intracellular water in cultured slices was approximately 50% smaller (0.70 to 0.37, no units). This difference could represent a decrease in overall cellular density or an increase in the astrocyte-to-neuron ratio in cultured rat hippocampal slices due to neuronal attrition during the culture process or to differences in cytosol water density between neurons and astrocytes. It also may reflect the absence of finely structured neuropil development in the embryologically younger cultured slice nervous tissue (postnatal day 9). The accelerated transmembrane exchange of water in cultured hippocampal slices ($67\ s^{-1}$) also may reflect a reduction in fine neuropil structures or it could be due to differences in membrane permeabilities. These differences may be explained largely by the significant cytoarchitectural heterogeneity of cultured hippocampal slices (Fig. 2) compared to the acutely prepared cortical slices. In fact, the mean intracellular diffusion coefficient and extracellular ADCs were not significantly different. This suggests that despite some differences in the intracellular fraction and exchange rate constant, the intracellular and extracellular environments sampled by water were nearly identical in acutely prepared and cultured brain slices. Thus, the data in this study indicate that slice cultures offer a reasonable model of nervous tissue microstructure for diffusion MRI investigations that is more biologically valid than other static tissue models such as erythrocyte ghosts (Thelwall et al., 2002).

Cultured rat hippocampal slices maintained excellent viability under the conditions required for MRI investigation for at least 4 h at room temperature even without perfusion or gas exchange. Further, this tolerance extended to cultured slices under magnet conditions at 35°C when slices also were incubated with 95% O_2/

5% CO_2. The changes observed with GFAP and NeuN immunohistochemistry suggested a moderate reactive gliosis and some mild neuronal shrinkage occurred in cultured slices after 4 h under MRI-like immersion conditions. However, it was notable that there was some baseline increased GFAP immunoreactivity (from the culture process), and the observed immunohistochemistry differences from increasing immersion time were only slightly greater than the magnitude of individual cultured slice variability. Nuclear pyknosis, a finding correlated with the initiation of apoptotic cell death cascades, was a rare observation in DAPI immunohistochemistry of cultured slices after 4 or 8 h of immersion. Also, phase-contrast microscopy did not indicate gross microstructure changes to the slice cultures even after 8-h incubation. These findings suggest that choosing healthy rat hippocampal slice cultures prior to the MRI experiment may best determine how long the samples can tolerate immersion conditions beyond 4 h.

Cultured slices tend to be more viable and tolerate longer periods without perfusion under magnet conditions than acutely prepared rat brain slices because they have already adjusted to Wallerian degeneration initiated during tissue procurement and their inherent thinness reduces problems from delayed diffusion of nutrients into the tissue core. Data from this study indicate that cultured rat brain slices may have limitations to the total time they tolerate imaging conditions even with perfusion. However, the main experimental advantage of cultured rat brain slices over acutely prepared brain slices is that they can survive for several weeks after procurement (Scheffler et al., 2003) and be re-cultured after initial MRI characterizations; this feature should allow studies of more chronic pathologic processes as well as characterizing cellular processes that occur over days or weeks. Also, without the strict necessity of perfusion, this setup can be simpler than methods for MRI of acutely prepared brain slices (Shepherd et al., 2002). There also is less chance of tissue movement during the MRI experiment because the slice cultures are adherent to a culture membrane that is firmly compressed between the brackets. This novel apparatus also enabled the first gradient echo images of brain slices by eliminating the polypropylene mesh (and its associated susceptibility artifacts) described in previous studies (Shepherd et al., 2002). Although the thinness of slice cultures creates additional difficulties to obtaining sufficient SNR, conversely, this thinness is more amenable to correlative techniques that may prove essential to certain MRI investigations of tissue microstructure (e.g., confocal microscopy) and facilitates higher throughput since more individual slice cultures can be stacked in the NMR tube within the sensitive region of the radiofrequency coil.

There are, however, some challenges when using cultured brain slices to model in vivo nervous tissue for MRI investigations. Proliferative astrocytes and microglia and reorganizing neuronal fibers may affect results obtained from cultured brain slices (Caeser and Aertsen, 1991; del Rio et al., 1991; Derouiche et al., 1993; Hailer et al., 1996). In addition, to use the method and device described here, appropriate infrastructure for derivation and maintenance of slice cultures as well as vigilant sterile techniques during the imaging period are required.

In summary, this study demonstrates that cultured hippocampal slices retain the 3-dimensional cytoarchitecture of in vivo nervous tissue, tolerate long imaging protocols in high-field, narrow-bore research magnets without movement, and by lacking a blood–brain barrier, permit significant experimental control over the extracellular environment. Cultured brain slices have several clear advantages over acutely prepared brain slices, including ease of use (without the necessity of perfusion), a better tolerance for physiological temperatures, and less swelling and acute pathology from procurement during data acquisition. Slice cultures can be grown for several weeks and may be re-cultured after imaging, which should prove useful for time-intensive MRI investigations of contrast mechanisms (e.g., diffusion) or delayed pathologic tissue reorganization. Although slice cultures are significantly thinner than acutely prepared brain slices, data from this study indicate that MRI of cultured slices at magnet field strengths lower than 14.1-T should be attainable. The thinness also makes slice cultures more amenable to correlative techniques such as confocal microscopy. In addition, gradient echo imaging of tissue slice cultures should enable the evaluation of molecular and intracellular contrast agents for molecular and stem cell MRI investigations. Each of these advantages may also be available to cultures of other tissue types (e.g., cardiac tissue).

Acknowledgments

The authors wish to thank Dan Plant for technical assistance with the magnet, Samuel Grant for helping with gradient echo imaging, Peter Thelwall for helping with figure preparation and Shanshan Wang for helping with tissue processing for immunohistochemistry. We are grateful for funding from the NIH (RO1 NS 36992, P41 RR16105, R21 NS46384, NIH/NINDS NS37556).

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