Cytoarchitectural Basis for Water Diffusion in Rat Hippocampal Slices

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INTRODUCTION

Diffusion MRI is a clinically useful surrogate marker of acute ischemia, yet the biophysical basis for the observed changes in water diffusion after ischemic brain injury remain poorly understood. Further investigations of water diffusion in nervous tissue are presently hampered in human subjects because of hardware limitations. Alternatively, brain slices can be used to model the water diffusion properties of *in vivo* nervous tissue well [1] and thus be used for time-intensive investigations required to develop better analytical models of water diffusion. However the previous studies averaged signal changes over the brain slice. In this study, an examination of signal heterogeneity within the brain slice is conducted and related to the tissue structure. Hippocampal slices offer an especially interesting tissue substrate for such studies because they possess many unique cytoarchitectural regions that can be segmented with high-resolution MRI.

METHODS

Rat hippocampal slices were imaged using a 17.6T wide-bore magnet with a 10-mm birdcage NMR coil as previously described [2]. Additional slices were procured for histological characterization with hematoxylin and eosin (H&E) for cell morphology or Weil's stain for myelin. All MRI data were obtained from 117-µm in-plane resolution images with 300-µm slice thickness. Scans lasted < 1 hr to allow sufficient ACSF perfusion for slice viability. A pulsed-gradient spin-echo multislice sequence with TR/TE = 2000/30s and a diffusion time (T_d) of 14 ms provided diffusion-weighted images with *b*-values between 0 and 8000 s/mm^2. The diffusion gradients (0 - 940 mT/m) were aligned with the read, phase or slice imaging-gradients to assess water diffusion anisotropy. A biexponential equation was fitted to diffusion-weighted signal attenuation using a nonlinear least squares fitting routine to give the fraction of fast diffusing water (F_{fast}), and the fast and slow apparent diffusion coefficients (D_{fast} & D_{slow}). T_1 and T_2 values were measured with a saturation recovery (TR = 150 ms – 10 s) and fast spin-echo sequence (TE = 10 ms, 30 echoes) respectively. After image acquisition, diffusion-weighted images (b > 3500 s/mm^2) were used to draw regions-of-interest (ROI) in the hippocampal slices; these included the whole slice, subiculum, CA1, CA3, stratum radiatum, stratum oriens, hilus, granule cell and molecular layers, as well as noise and perfusate. The biexponential diffusion parameters, T_1 and T_2 values for the different ROIs then were compared statistically using a 1-way ANOVA with posthoc Tukey multiple-comparisons tests.

RESULTS

For clarity, figure 1 shows diffusion-weighted signal attenuation curves only for ROIs in the dentate gyrus of the rat hippocampal slice, but similar dispersion was noted for all ROIs. Individual comparisons between ROIs demonstrated many statistically significant differences (N = 20, P < 0.05) for the biexponential diffusion parameters F_{fast} and D_{slow} that appeared independent of diffusion-gradient orientation (3 orthogonal gradient directions, N = 10). To simplify analysis, the hippocampus can be divided into 3 cytoarchitectural tissue types based on H&E histology; large, densely packed neuronal bodies (type 1: CA1, CA3 & granule cells)(Fig. 2A), neuropil or densely interdigitated axons and dendrites (type 3: molecular layer, stratum oriens &radiatum) (Fig. 2C), and mixed neuropil and neuronal bodies (type 2: hilus & subiculum) (Fig. 2B). Based on this schema, we observed the following differences in water diffusion; 1) F_{fast} decreased by 20% from type 1 to type 2 tissue and by 20% again from type 2 to type 3, 2) D_{slow} was 70% greater in type 1 tissue than types 2 or 3. Unlike the diffusion differences noted, the ROIs did not have T_1 and T_2 differences (N = 5, P > 0.05). Myelin distribution also did not show substantial regional differences. Nor did H&E-stained sections of slices demonstrate particular ROIs (e.g. CA1) exhibiting selective vulnerability to injury from slice procurement or perfusion in the magnet. Segmentation of H&E-stained sections suggested the MRI volumes of the CA1, CA3 and granule cell layer ROIs may have been overestimated by 2-3 times (Fig. 2D), even when shrinkage due to tissue processing was accounted for by normalization.

DISCUSSION

This study demonstrates statistically significant differences in water diffusion for unique cytoarchitectural regions of the rat hippocampal slice; these differences appear independent of regional anisotropy, selective vulnerability, mylein content, T_1 or T_2 differences. The biexponential model is underparameterized, but changes in F_{fast} (and D_{fast}) may correlate with changes in the relative size of the intracellular compartment [4,5] and changes to D_{slow} may correlate with changes in intracellular restriction [5]. F_{fast} increases significantly with decreases in neuropil content. This could be related to decreases in intracellular fraction [6], but is surprising since the regions with highest F_{fast} are composed of large, densely-packed neuron cell bodies (Fig. 2A). Alternatively, the increased tortuosity of the extracellular space due to the complex interdigitation of axons and dendrites in the neuropil may decrease F_{fast}. It is also possible that water transmembrane exchange rates differ because of structure-function differences of cytoarchitectural regions. D_{slow} was 70% faster in type 1 tissue and may explained by the differences in cell size for large pyramidal neurons (diameter >10 µm) versus the thin processes of neuropil (diameter < 1µm) present in tissue types 2 and 3. The differences in F_{fast} and D_{slow} are especially intriguing since histology suggests significant partial volume effects in type 1 tissues – perhaps 60% of diffusion-weighted signal in these ROIs was from adjacent layers (Fig. 2D). This suggests the differences described here may be significantly underestimated. Ongoing studies aim to examine differences in water exchange time, and the intra- or extracellular T_1 and T_2 values for various regions of the hippocampal slice.

REFERENCES

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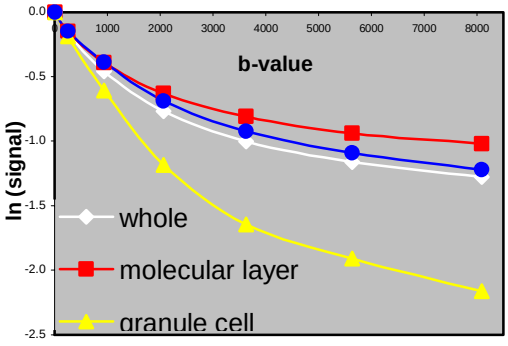

Figure 1 – Diffusion-weighted signal attenuation curves in the different regions of the dentate gyrus and whole hippocampal slice.

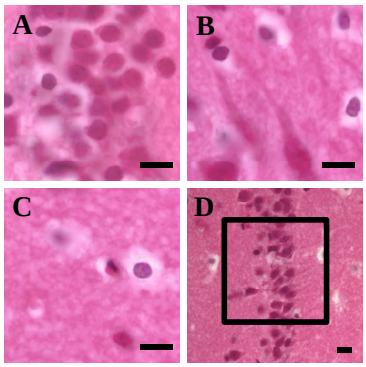

Figure 2 – H&E of the granule cell layer (A), hilus (B) and molecular layer (C). Panel D depicts MRI voxel dimensions on the CA1 region of the rat hippocampus [bars = 10 µm].