Relaxation, Magnetization Transfer, and Diffusion Tensor Measurements in the Hippocampal Formation of APP and PS1 Transgenic Mice

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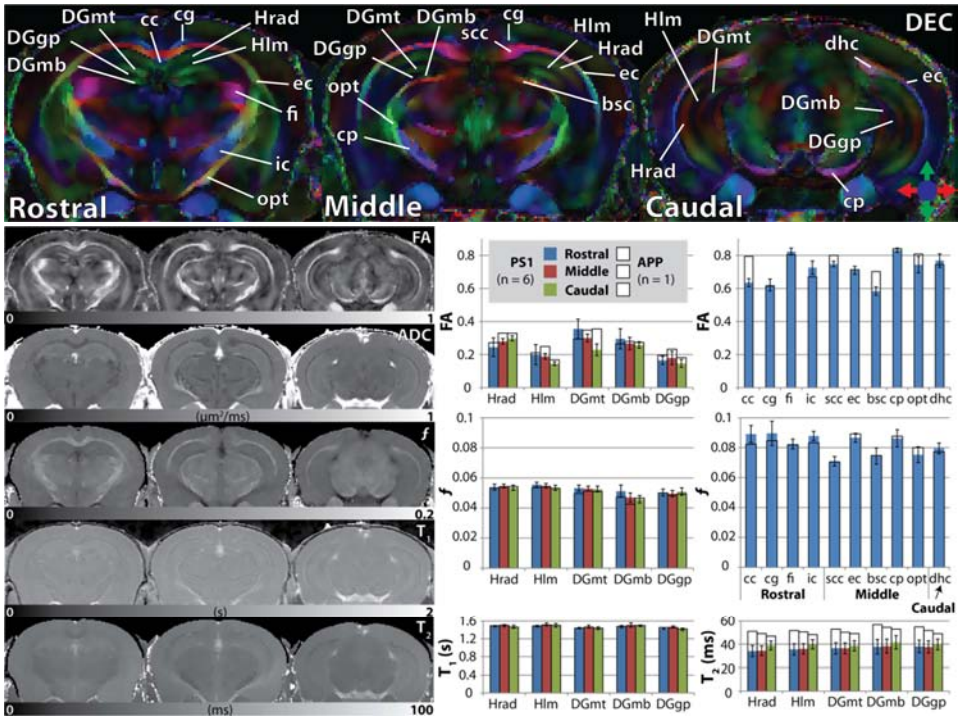
Introduction Alzheimer's disease (AD) results in atrophy and microstructural changes in the hippocampus with peripheral damage to various white matter (WM) structures[1,2]. MRI methods capable of quantifying structural changes in the hippocampal formation and surrounding WM can improve both the diagnosis and understanding of AD. Although several studies have applied quantitative magnetization transfer imaging (qMTI)[3-5] and diffusion tensor imaging (DTI)[2,6-8] in and around the hippocampal formation, studies focused on different structures within the hippocampus itself have been limited primarily to DTI[9-10]. The qMTI-derived bound pool fraction (ƒ) and water fraction of the short-T_2 component (MWF) might show changes in tissue content beyond just the myelin content with which they are often associated. In this study, T_1 relaxation, multicomponent T_2 relaxation, DTI, and qMTI were used to analyze the hippocampal formation and surrounding WM structures in seven *ex vivo* transgenic mouse brains either overexpressing the presenilin-1 (PS1) protein (n=6) or the amyloid precursor protein (APP) (n=1).

Methods **Mouse Model:** Mice were perfused with PBS followed by 4% PFA at approximately 7 months of age. Brains (in skull) were transferred to a PBS solution 48 hours prior to imaging to leach out any remaining PFA. All experiments were approved by the university's animal care committee. **MRI:** Using a 7 T Bruker Avance III NMR system with Paravision 5, 3 coronal slices (0.5 mm slice thickness, 1 mm interslice distance, (2 cm)^2 FOV, 256x256 matrix size) were selected in each mouse to span the hippocampal formation. **Relaxation Imaging:** T_1 maps were determined by fitting a saturation recovery curve to 5 RARE images (effective T_E = 11 ms; T_R = 4895.5, 2895.5, 1395.5, 695.5, 295.5, 95.5 ms; RARE factor = 2; 4 averages; 71 minutes). Multicomponent T_2 maps were determined using a NNLS algorithm with 120 logarithmically spaced signal amplitudes and T_2 values from 5 – 500 ms applied to a CPMG acquisition with 32 slice-selective π-pulses and a 10 ms echo spacing (T_R = 2500 ms, 12 averages, 96 minutes). Myelin water fractions were calculated for signal amplitudes of T_2 < 20 ms, as was the geometric mean of the intra-/extra-cellular water component (20 ms < T_2 < 100 ms), reported here as just "T_2". **qMTI:** Quantitative MT maps were determined using an approximation of the two-pool model of magnetization tranfer[11] fit to 1 non-saturated and 18 RF-saturated FLASH images (10.25 ms Gaussian saturation pulse with saturation powers of 5, 10, and 20μT and frequency offsets at each power of 1000, 2000, 4000, 6000, 10000, and 30000 Hz, 32 averages, T_E = 6 ms, T_R = 70 ms, 10° flip angle, 9.5 minutes/image) **DTI:** DTI maps were determined using a non-linear least squares fit and a modified Cholesky decomposition to ensure positive definiteness[12] fit to a PGSE acquisition (7-direction tetra-orthogonal gradient-encoding scheme, b-value = 1000 s/mm^2, δ = 6 ms, Δ = 14 ms, TE 26ms, TR 5000ms, 6 averages, 8.5 hours). **Image Analysis:** Prior to fitting, all images were aligned to the b=0 diffusion-weighted images using a rigid affine transformation matrix determined automatically by maximizing the 2D correlation coefficient. An anisotropic diffusion filter was applied with 5 iterations and a gradient modulus threshold equal to 3 times the standard deviation of noise in each image[13]. ROIs were selected in the directionally encoded color map (DEC) with reference to a mouse atlas[14] and applied to analysis of all quantitative MR maps.

Results and Discussion Anatomical details visible only in DTI anisotropy maps allowed regions of interest to be defined and applied to all quantitative MRI maps. Fractional anisotropy (FA) and the FA-weighted DEC maps had much better contrast within the hippocampal formation when compared to other quantitative MR methods. FA varied significantly in different regions of the hippocampal formation and surrounding WM structures. MWF ranged from 0 to 0.10±0.05 in different WM regions, but was effectively 0 in all hippocampal regions. T_1 and T_2 were also fairly uniform across the hippocampal formation. ƒ was lower in the hippocampus and dentate gyrus, but with little variation compared to the different WM regions. The forward magnetization exchange rate (RM_0^B), which recently was shown to be reduced in the hippocampus of patients with AD[5], was lower in regions of the hippocampal formation (1.37±0.13 to 1.68±0.15 s^{-1}) when compared to the various WM tracts (1.76±0.07 to 2.24±0.08 s^{-1}). Although our results reveal regional differences, further comparison with age-matched controls is required to determine the efficacy of different quantitative MRI methods when applied to the study of APP and PS1 mouse models of AD

Conclusion Quantitative MRI methods such as DTI and qMTI are useful for determining changes in tissue structure and content within the hippocampal formation and surrounding WM tracts of APP and PS1 mouse models of Alzheimer's disease. This work lays the foundation for future studies, which will incorporate *in vivo* and longitudinal measurements in transgenic mouse models of AD alongside age-matched controls and correlation with pathohistology.



Figures **Top:** ROIs were selected in the hippocampus, dentate gyrus, and various WM tracts surrounding the rostral, middle and caudal regions of the hippocampal formation. **Bottom Left:** Representative quantitative MRI maps from a PS1 mouse. **Bottom Right:** Select results from PS1 and APP mice. FA shows variations in the hippocampal formation. Both FA and ƒ are elevated in WM, as expected.

ROIs **Hippocampal Formation:** radiatum (Hrad) and lacunosum-moleculare (Hlm) layers of the hippocampus; top/bottom molecular (DGmt/DGmb) and granular/polymorph (DGgp) layers of the dentate gyrus. **WM:** corpus callosum (cc), cingulum (cg), fimbria of the hippocampus (fi), internal capsule (ic), splenium of cc (scc), external capsule (ec), brachium of the superior colliculus (bsc), cerebral peduncle (cp), optic nerve (opt), and dorsal hippocampal commissure (dhc).

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