Unique pattern of diffusion metrics sheds light on cellular changes during hypoxic-ischemia

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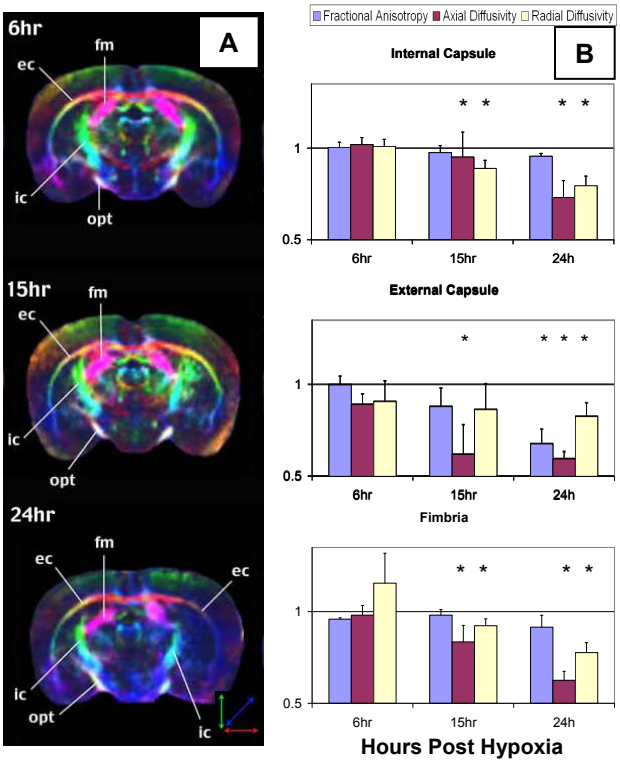
Introduction: Diffusion tensor magnetic resonance (DTMR) imaging is a powerful tool for elucidating micro-architectural structure in white matter (WM). Leukoaraiosis, the rarefaction of WM, was originally coined to describe hyperintensities in WM observed in T2 weighted MRI images, and has been correlated with increases in the risk of stroke and post stroke dementia [1]. In this study, we use an animal model for leukoaraiosis to determine whether DTMR can distinguish characteristics unique to this pathology.

Methods: We use a modified Levine/Vannucci procedure (unilateral carotid artery ligation followed by transient, systemic hypoxia) with tight body-temperature control to increase the consistency of brain damage in adult rodents [2]. This model mimics the oxidative stress and small-vessel hypoperfusion found in leukoaraiaosis. Animals were sacrificed at 6, 15, and 24 hours post-hypoxia. *Ex vivo* DTMR microimaging was performed followed by electron microscopy (EM) to aid in the interpretation of our DTMR data. DTMR images were collected on a 7 Tesla Bruker Avance System equipped with 400 mT/m actively shielded gradients. All *ex vivo* images were acquired with a custom-built solenoid transmit/receive coil. Whole brain data were collected using a 3D, conventional Stejskal-Tanner single spin echo sequence with diffusion sensitizing gradients along six directions with the following parameters: FOV=32x12x12mm^3, matrix=256x96x96, resolution=125 μm isotropic, b=800 sec/mm^2, gradient separation/duration (Δ/δ)=12/4 ms, and TE/TR=22/1000ms. Quantification of the anisotropy measures—fractional anisotropy (FA), axial diffusivity ($\lambda_{\parallel}$), and radial diffusivity ($\lambda_{\perp}$), was performed using DTIstudio software [3]. Regions of interest were drawn in the external capsule, internal capsule, and fimbria of the hippocampus from multiple slices spanning the majority of those structures.

Results and Discussion: We found noticeable changes in WM as early as 6 hours post hypoxia. Contrary to the common finding of decreased FA due to decreased $\lambda_{\parallel}$ and increased $\lambda_{\perp}$ in previous stroke studies [4], we observed a trend toward decreasing $\lambda_{\parallel}$ *and* $\lambda_{\perp}$ in all three WM structures. This resulted in an insignificant change in FA values as illustrated most dramatically in the internal capsule. In previous studies, demyelination caused an increased $\lambda_{\perp}$ [5]. However, in our leukoaraiaosis model, hypoxia-ischemia caused separation of myelin sheaths, which resulted in the protrusion of vesicles from the myelin and compression of the axon plasma. Furthermore, the damage to the myelin sheets created multiple compartments to confine diffusion and led to a reduced $\lambda_{\perp}$. These results indicate the possibility of differentiating types of cellular damage as a consequence of hypoxic-ischemia using DTMR.

Conclusion: When $\lambda_{\parallel}$ decreases and $\lambda_{\perp}$ increases following axonal damage and demyelination, FA values will decrease signaling WM damage. However, in our leukoaraiosis model WM damage was reflected by decreases in *both* $\lambda_{\parallel}$ and $\lambda_{\perp}$. Our observations suggest that $\lambda_{\parallel}$ is a more sensitive and reliable metric than FA in detecting WM injury in this type of stroke-related hypoxic-ischemic brain injury.



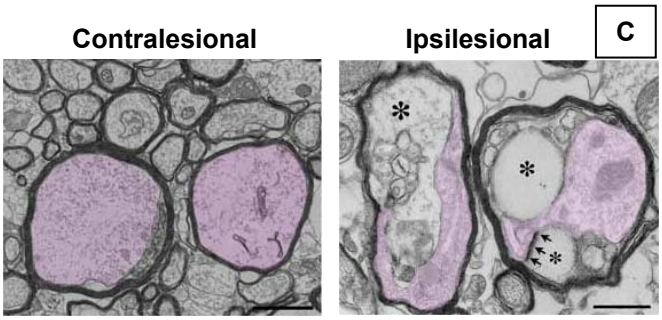


Figure 1 **A**: Direction-encoded-colormap showing evolution of WM damage. **B**: Changes in the FA, $\lambda_{\parallel}$, and $\lambda_{\perp}$. Vertical axes represent normalization of ipsilesional with respect to contralesional hemisphere, n=9 total, 3/time point, * = $p < 0.05$ and is based on one way ANOVA with respect to 6hr time point. **C**: EM of external capsule at 24 hours. The myelin is still intact, however, the innermost myelin sheet layers separate (arrows); the mitochondria swell, and multicompartmented intercellular spaces are created (*) that compress the axoplasma (pink). These biophysical mechanisms suggest an explanation for the reduction in $\lambda_{\perp}$. Scale bar = 1μm.

References: **1** Hachinski VC, et al. Arch Neurol, 1987;44:21-23. **2** Adhami F, et al. Am J Pathol, 2006;169:566-583. **3** Jiang H, et al. Comp Met Prog Biomed;81:106-116. **4** Wang S, et al. Stroke, 2008;39:2348-2353. **5** Harsan LA, et al. J Neuro Res, 2006;83:392-402.