Diffusion Tensor Imaging of ALS-PDC at 21.1 T: *in utero* vs *ex utero* exposure

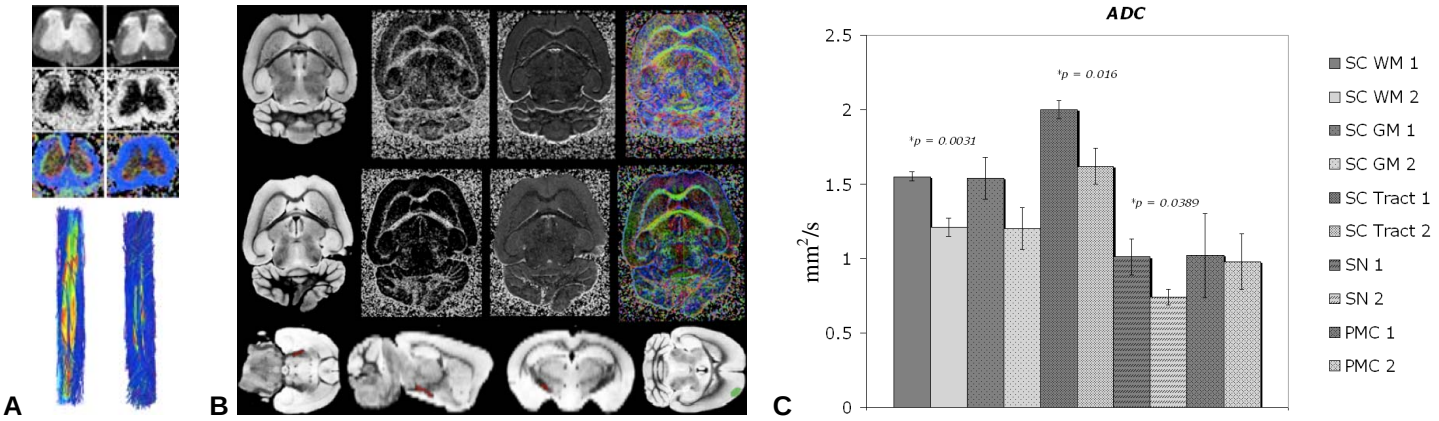
T. M. Gould[1,2], J. T. Rosenberg[1,2], I. S. Masad[1,2], Y. Banjo[3], C. A. Shaw[3,4], and S. C. Grant[1,2]

[1]Chemical & Biomedical Engineering, Florida State University, Tallahassee, FL, United States, [2]National High Magnetic Field Laboratory, Tallahassee, FL, United States, [3]Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada, [4]Ophthalmology, Physiology, and Experimental Medicine, University of British Columbia, Vancouver, BC, Canada

INTRODUCTION: Amyotrophic Lateral Sclerosis (ALS) is an invariably fatal, chronic and progressive neurodegenerative disease that affects the descending motor neurons of the central nervous system with primary white matter involvement. Previous findings have revealed an etiological component—purified sterol β-D-glucoside (BSSG), a neurotoxin extracted from seeds of *Cycas circinalis* (a palm tree local to Guam)—that induces a hybrid pathology, ALS Parkinsonian Dementia Complex (ALS-PDC). This disease displays symptoms of ALS-related muscular dysfunction, as well as Parkinsonian tremors and cognitive dementia [1]. Because this pathological agent can be ingested, there is the potential for neurodegeneration to occur early in development, even though these deficits may not become apparent until later in life. In this study, the effectiveness of the placental barrier to BSSG was examined by administering the purified sterol *in* and *ex utero*. Excised, fixed brains and spinal cords for the treated offspring were analyzed by diffusion tensor imaging (DTI) at 21.1 T.

METHODS: Fixed mouse brain and spinal cord specimens were taken from two groups (n=3): (1) an *in* and *ex utero* treatment group that was exposed to BSSG both prior to and after birth and (2) an *in utero* treatment group that was exposed to BSSG only in the womb. MR Microscopy (MRM) was used to generate 25-µm isotropic resolution anatomical datasets using a true 3D gradient-recalled echo sequence. DTI data were acquired through the use of a multi-slice 2D spin echo sequence at a resolution of 40x40x400 µm for spinal cords and 50x50x250 µm for brains. The tensor representing water diffusion was sampled in seven directions, with three diffusion weightings (0, 500 and 1000 s/mm^2) per direction [2]. All data were processed using MedINIRA, an image processing and DTI software package developed as part of the Asclepios research project [3]. Maps of the apparent diffusion coefficient (ADC), fractional anisotropy (FA) and primary eigenvalues (λ_1, λ_2, λ_3) were generated. Additionally, using voxel-by-voxel assessments of FA, whole volume fiber tractography was performed on spinal cords to visualize connectivity changes between treatment groups [4]. For statistical analysis, manually drawn ROIs delineated white and gray matter lumbar areas of spinal cord, substatia nigra and primary motor cortical areas.

RESULTS: B_0, FA, color-FA and whole volume fiber tractography data of an excised mouse spinal cord sample from each group are shown in Fig. A. Fig. B compares B_0, FA, ADC and color-FA maps between brain samples from each group. ROI and fiber tractography ADC averages were pooled, and significant differences (from two-tailed t-test, $p<0.05$) between groups with respect to ADC in spinal cord white matter and substantia nigra are presented. In Fig. C, statistics reveal that Group 1 spinal cord white matter, fiber tracts and substantia nigra exhibit significantly higher ADCs than Group 2. Spinal cord gray matter and primary motor cortex ROIs report a high ADC trend for Group 1 compared to Group 2; however, these results are not deemed significant. No significant differences in FA were found between groups.



***Figure(s)**: Fig. A: B_0 (top), FA, color-FA axial images and whole volume fiber tractography (bottom) of excised spinal cords for Group 1 (left column) and Group 2 (right column). Fig. B: (from left to right) B_0, FA, ADC, and color-FA for Group 1 (top row) and Group 2 (middle row), and ROI selections (bottom row) for substantia nigra (red) and primary motor cortex (green). Fig. C: Pooled Group (1/2) ADC values for spinal cord (SC), white matter (WM), gray matter (GM), fiber tracts, substantia nigra (SN), and primary motor cortex (PMC). *Denotes significance.*

DISCUSSION: Higher ADCs for Group 1 compared to Group 2 predict that Group 1 ALS-PDC models are more severely affected by the disease than Group 2. In Fig. A, the FA images of Group 2 spinal cord (right) render a 'complete' white matter structure, whereas FA images from group 1 (left) show an incomplete white matter. Tractography data displays axonal integrity; though not significantly different, overall quantification yielded more fibers present for Group 2 compared to Group 1. In general, mainly high ADC values characterize the effects of Group 1 *ex utero* treatment. Higher ADC can be interpreted as less restricted diffusion inside tissue; therefore, axonal disruption—possibly from demyelination—may be present to a greater extent in Group 1.

CONCLUSIONS: The hypothesis that the placental barrier may partially inhibit/slow neuropathogenesis has been explored via DTI in the ALS-PDC model. At 21.1-T, DTI shows that ADC measurements within excised mouse central nervous system can distinguish a subsequent *ex utero* treatment of the sterol BSSG. These findings predict that *in utero* neurotoxicity resulting from BSSG is less harmful to the developing fetus, whereas a higher-grade neuropathogenesis may present if there is BSSG exposure after birth.

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