Magnetization Transfer Ratio Differences in the Adult Mouse Brain Due to Cranial Irradiation in Infancy

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Target Audience: Our target audiences are clinicians and scientists interested in the effects of irradiation on development, as well as those who are interested in the quantification of white matter abnormalities.

Introduction: Neurocognitive sequelae due to therapeutic cranial irradiation, such as memory loss and reduced intellectual functioning, are most severe when treatment is performed at a young age [1]. Unfortunately, the mechanisms by which these sequelae occur are poorly understood. Previous research has demonstrated that children [1] and young mice [2] that were treated with cranial irradiation have significantly reduced white matter (WM) volumes later in life, detectable with conventional MRI. Magnetization Transfer (MT) imaging is an MR technique that can be used to detect differences in macromolecular content and is frequently used to asses WM [3]. The objective of our study was to identify WM differences in the mouse brain due to early postnatal cranial irradiation, detectable through the use of MT imaging.

Methods: Animals: Twenty-two female C57Bl/6J mice were exposed to a single dose of 7Gy (equivalent to 16Gy in 2Gy fractions) whole brain irradiation at postnatal day (P)16, corresponding to the infant stage in the human. All mice were sacrificed at P63, approximately equivalent to early human adulthood. **MRI:** MT reference images were acquired *ex-vivo* on a 7T scanner (Agilent Technologies Inc.) using a 3D gradient echo sequence; 98µm isotropic resolution, 4 averages, TR/TE=300/4ms, 296x184x184 matrix size, and 68° flip angle. Immediately following this, MT images were acquired using the same sequence, starting with an MT saturation pulse (+3500Hz off resonance, 20ms long, Gaussian shaped). Complete acquisition of both reference and MT images took ~ 14.5hrs. **Image Analysis:** The magnetization transfer ratio (MTR) was calculated as the percentage reduction in voxel intensity from the reference to the MT image, with a correction for average cerebral cortex MTR. An automated registration process was used to align the reference images to a consensus average; the same deformation was applied to each MTR image. 62 automatically-segmented brain structures were aligned with the consensus average and then back projected onto each individual MTR image. Mean MTR was calculated for each segmented structure. Statistical calculations were performed using R (www.r-project.org) and p-values were corrected for multiple comparisons through the false discovery rate (FDR) method.

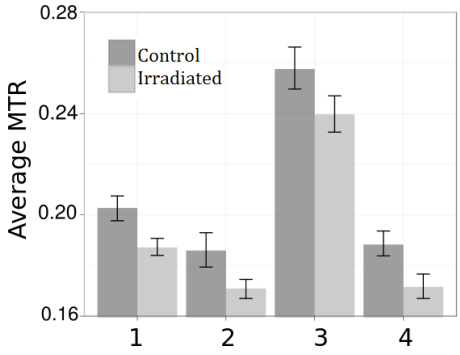

Figure 1. Average MTR value for irradiated and control brains in the (1) anterior commissure: pars anterior, (2) anterior commissure: pars posterior, (3) cerebral peduncle and (4) fornix.

Results: A significant difference in mean structure MTR, between irradiated and control groups (20%FDR, unpaired t-test), was detected in the anterior commissure, fimbria and cerebral peduncle (**Figure 1**). Significant differences (20%FDR, unpaired t-test) in parts of the corpus callosum were also detected by voxelwise comparison (**Figure 2**). Analysis of the affected area of the corpus callosum with H&E staining shows that WM in the irradiated brain has a disrupted organization compared to the control (**Figure 3**).

Discussion and Conclusion: The current study demonstrates that MT imaging can be used to identify differences in WM of the developed mouse brain due to cranial irradiation in infancy. Although white matter lesions are detectable with conventional T2-weighted MRI, MT imaging provides a quantitative method for identifying pathological differences such as dymyelination and axonal loss that the former cannot [4]. Future work will aim to identify the cellular mechanisms behind neurocognitive sequelae using transgenic mice. Application of MT imaging to this research will quantify the WM outcomes in these strains.

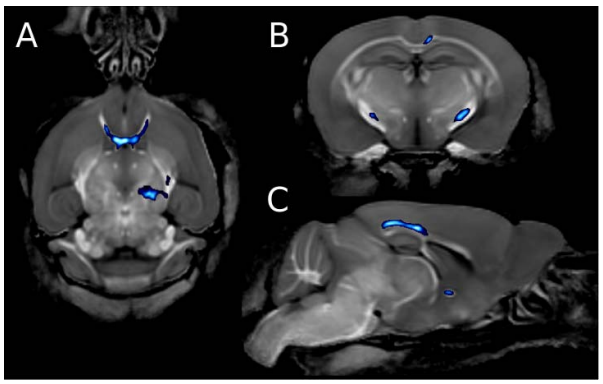

Figure 2. (A) axial, (B) coronal and (C) sagittal slice from an MTR image of the average developed brain. A colour map has been overlaid indicating areas where the MTR is significantly less in irradiated mice than in controls.

References: [1] RK Mulhern et al. (2003) Lancet Oncol. **[2]** LM Gazdzinski et al. (2012) Int J Radiat Oncol Biol Phys. **[3]** RM Henkleman (2001) NMR in Biomedicine **[4]** K Schmierer (2004) Annals of Neurology

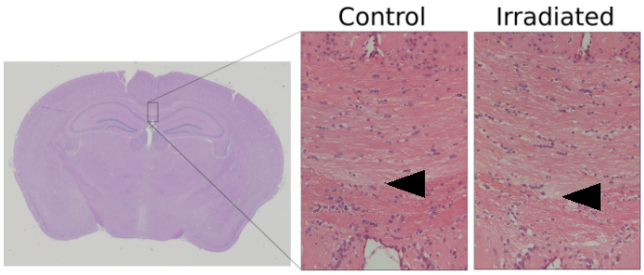

Figure 3. Coronal slice of a control brain stained with H&E and a zoomed in section of the corpus callosum for both a control and irradiated brain. Arrow indicates boundary location.