Alterations in brain development induced by whole-brain irradiation in young mice

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Introduction

Acute leukaemia and brain tumours are the two most common childhood cancers. As long-term survival rates continue to improve, late side effects are of increasing concern[1]. The standard of care for both cancers includes cranial radiotherapy, which has been linked to progressive neurocognitive dysfunction, most often measured as a decrease in IQ. While certain structures, including the neurogenic niches of the hippocampus and subventricular zone are considered radiosensitive[2], and cognitive deficits correlate with a decrease in normal-appearing white matter volume[3], a systematic mapping and longitudinal evaluation of structural alterations in the developing brain after irradiation has not been performed. The goal of the current work is to identify regions of the developing mouse brain that are most sensitive to radiation as a step towards modifying current treatment regimes to mitigate the late effects of paediatric radiation treatment of the brain.

Methods

Under anaesthesia, female C57Bl/6 pups (n=20) received whole-brain irradiation to a dose of 7Gy from a ^{137}Cs source at 2.5 weeks of age. The rest of the body was shielded by 3.2cm of lead, restricting doses there to ~0.4Gy. Control mice (n=19) were also irradiated, but placed entirely beneath the shielding. *In vivo* MRI at 7T using a 3D gradient echo sequence (125µm isotropic resolution, TR/TE/α=100ms/4ms/55°) was performed prior to irradiation at 2 weeks of age, and then following irradiation at 3.5, 6, and 9 weeks of age. At all imaging time points, mice were administered 0.4 mmol/kg $MnCl_2$ intraperitoneally 24 hrs prior to imaging.

The images from all time points were registered together to generate a consensus average. The Jacobian determinants from the deformation fields, which define the voxel displacements from each image to the average, were used to obtain local volume differences between each image and the average, enabling a voxel-by-voxel volume comparison between the irradiated and control groups. Statistical analysis of the data was performed using a linear mixed effects model that allowed different growth rates for the two groups and random intercepts for individual mice. Absolute brain structure volumes were also calculated for each image using a segmented atlas with 42 labelled structures. Multiple comparisons were controlled for using the false discovery rate (FDR).

Results

Figure 1 summarizes the results of a voxelwise comparison of growth rate between irradiated and control mice. Colour maps indicate regions that show significantly slower growth in the irradiated group with greater significance represented by hot colours. Significant differences were seen bilaterally throughout the brain in white matter regions including the corpus callosum (coronal slice 2), fimbria (coronal slice 3), and cerebral peduncles (coronal slice 4), as well as in gray matter regions including the olfactory bulbs (coronal slice 1) and hippocampus (horizontal slice).

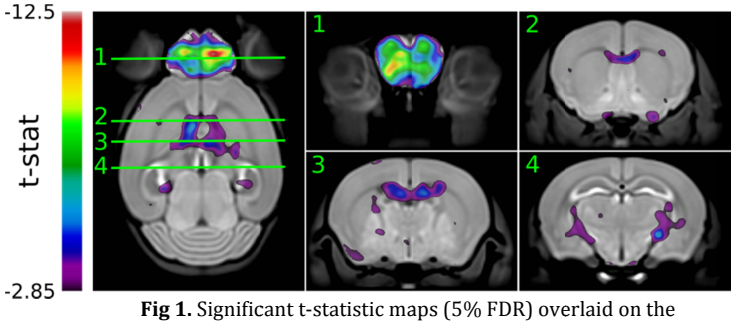

Fig 1. Significant t-statistic maps (5% FDR) overlaid on the consensus average image.

Figure 2 shows the resulting brain structure volumes (normalized to controls) that are significantly different in the adult (9 weeks). Longitudinal evaluation of the structure volumes revealed significantly smaller volumes in 11 structures at the 3.5-week time point, including the anterior commissure, dentate gyrus and fimbria. The number of significant structures increased to 28 by 6 weeks, but decreased to 13 structures by 9 weeks. This pattern suggests a progressive developmental defect that is at least partly resolved in a subset of structures.

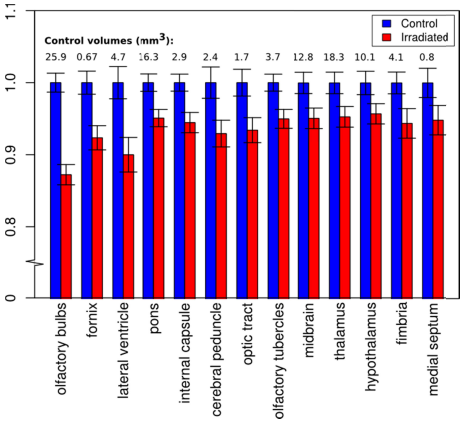

Fig 2. Bar plot showing brain structure volumes relative to the controls at 9 weeks (5% FDR).

Discussion and Conclusion

Cranial irradiation has been implicated in the development of neurocognitive late effects in childhood cancer survivors, but the mechanism remains poorly understood. Using longitudinal *in vivo* MRI, this study identified regions in the developing mouse brain affected by a single dose of radiation at an infant-equivalent age (2.5 weeks). Irradiation led to bilateral decreases in the growth rate and volume of both white and gray matter structures. The developmental time course varied somewhat among brain structures, with some structures showing changes at the first imaging time point (childhood), but most affected structures showing a progressive defect, becoming significant in adolescence (6 weeks). While some structures appeared to recover by adulthood (9 weeks), many remained smaller in the irradiated group through all time points examined. The systematic approach used in this study allows for longitudinal characterization of developmental alterations after cranial irradiation and may serve as a valuable tool for investigating neuroprotective strategies to mitigate late effects.

References: [1]Mulhern & Palmer, Curr Probl Cancer (2003) [2]Wong & Van der Kogel, Mol Interv (2004) [3]Mabbott et al., Neuro Oncol (2006)