IN VIVO CHARACTERIZATION OF MORPHOLOGICAL CHANGES IN PRENATALLY IRRADIATED MICE USING MRI

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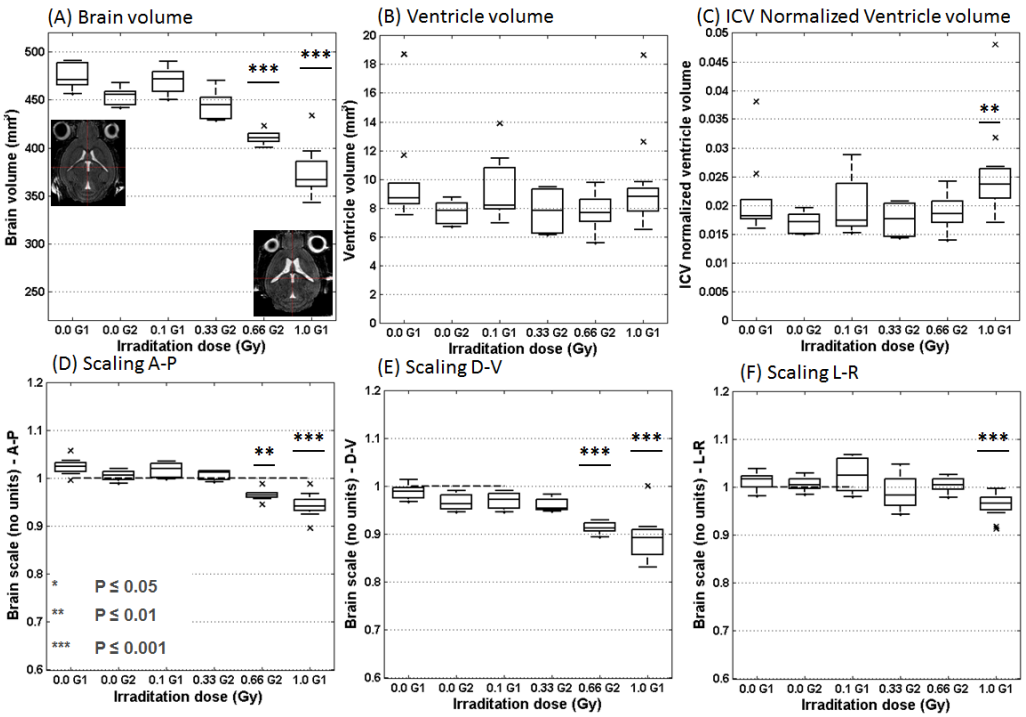
INTRODUCTION: Epidemiological studies have shown that *in utero* irradiation exposure causes a higher incidence of neurological disorders and cognitive defects like seizures and mental retardation [1]. For instance, studies on survivors of the atomic bombings of Hiroshima and Nagasaki demonstrated that prenatal radiation can have negative effects on adult brain functions. This was especially observed when exposure occurred between weeks 8 and 15 *post conception*, corresponding to approximately embryonic day (E)12 in mice [2]. These human studies contributed to the growing presumption that even low doses of radiation can cause long-term neurological defects. However, animal studies in this field are scarce and underpinning causes remain unclear. The main objective of this study is to characterize the morphological effects of *in utero* exposure to low and moderate doses of ionizing radiation on the mouse brain. Quantifying morphological changes through manual delineation of MR images is time-consuming, tedious, and requires trained expert. Alternatively, automated volume-of-interest (VOI)- based or voxel-based morphological analysis capture global and regional changes. In this work, we quantified the *in vivo* morphological differences among irradiated animals using a semi-automated regional segmentation and a VOI-based MRI morphometry approach. Morphological features of the mouse brain (e.g. ventricle volume, brain volume) that are related to cognitive functioning were investigated.

METHODS: At E12, pregnant C57Bl/6J mice were irradiated with different doses of X-rays (between 0.0 and 1.0 Gy, N=50) using a Pantak HF420 RX instrument operating at 250 kV, 15 mA, 1 mm Cu-filtered X-rays (dose rate of 0.375 Gy/min). The day of mating was counted as E1. After the irradiation, pregnant mice were placed back in their home cage in order to give birth to their offspring. Only female offspring was used in further experiments. Two subgroups of animals (G1, G2), with different dose conditions, were imaged at the age of 20 weeks with a 9.4 Tesla Bruker Biospec Scanner (Bruker Biospin, Ettlingen, Germany with following parameters: 3D RARE, TR=1300 ms, TE=14.2ms, matrix 192×256×128, 80µm resolution (isotropic). For morphological characterization, a semi-automatic small animal image analysis pipeline [3], which sequentially corrects for RF inhomogeneity, pose variations and inter-scan intensity variations was used. The MR image of a control animal (0.00 Gy) that yielded good spatial alignment (visually verified) was chosen as a representative image. The remaining study images were automatically registered to this representative MR image using maximization of mutual information similarity measure [4]. The manual delineations of the whole brain for the representative control animal were used as reference volume to determine an approximate relative volume measure for each of the study images. The registration-based volume measures (in mm^3) and scaling parameters were compared among different experimental groups. In a separate step, the ventricle volume of the study population was automatically determined using an intensity-based watershed segmentation method [5] The results of ventricle segmentation were visually examined for consistency and were manually corrected in a few cases. Volume measures of the whole brain and the ventricles were compared using a one-way ANOVA with Tukey for *post-hoc* comparison (significance level of 0.05).



RESULTS: The brain volume and ventricle volume (of the lateral and third ventricle) of 50 animals from the two subgroups G1 and G2 are reported in the Figure. For both subgroups, the brain volume of prenatally irradiated mice decreased significantly when compared to their respective controls . The reduction in brain volume was most evident for the higher dose conditions, i.e. a decrease of 9% and 22% for 0.66 Gy and 1.0 Gy, respectively ($p<0.001$). In addition, the brains of mice irradiated *in utero* appeared smaller along the anterior-posterior (A-P, head-feet), dorso-ventral (D-V, rostral-caudal) and left-right (L-R) axes (see Figure (D-F); $p<0.001$ for all axes). Similar comparison of ventricle volume changes showed no significant difference between different conditions. Although, when ventricle volumes were normalized with the respective intra cranial brain volumes (ICV) from Figure (A), there is trend of a large ventricle volume for highest irradiation dose (1Gy, $p<0.01$). This most likely indicates an enlargement of the ventricles of prenatally irradiated mice. It is important to note that due to a large heterogeneity in the ventricle volume between animals for the experimental and control groups (see outliers in (B)), observations on ventricle volume were not prominent across all animals. The above morphological changes are in line with altered spontaneous activity, reduced anxiety-related behavior and changes in higher cognitive functions, previously confirmed by behavioral experiments in the same animals [5].

DISCUSSION & CONCLUSIONS: The reduction in brain volume showed by *in vivo* MRI corresponds to the observation of a generalized and/or localized growth retardation (microcephaly) as in atomic bomb survivors [2]. Additionally, an enlargement of brain ventricular volume might attribute to changes in cognitive function assessed in a previously performed behavioral test battery with the same animals [6]. Although there seems to be a high variability within conditions, general dose-dependent trends in both brain and ventricle volume could be observed. Given the global shape changes noted in scaling parameters, future work could investigate other likely regional developmental alterations (e.g. volume changes in the cerebellum) and correlation of *in vivo* measurement with *ex vivo* histology.

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