EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

ESRF	**Experiment title:** Study of the early-stage effects of microbeam radiation therapy on rat brains by phase contrast micro-CT	**Experiment number**: MD1219
Beamline: ID17	**Date of experiment**: from: 16.04.2021 to: 17.04.2021 from: .04.2021 to: 17.04.2021	**Date of report**: 04.03.2022
Shifts: 9	**Local contact(s)**: Alberto Bravin	*Received at ESRF:*

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Report:

- **Objective of the proposal**

In this experiment, we aimed at using X-ray phase-contrast micro-CT (PCI-microCT) for post-mortem imaging of healthy brain neuro-morphology after in-vivo treatment with synchrotron generated X-ray Microbeam Radiation Therapy (MRT), a spatially fractionated experimental high-dose radiotherapy/radiosurgery modality. The goal was the completion of successful experiments realized at the ESRF on the same type of novel radiotherapy approach (MD1121, MD1037) on both healthy and glioblastoma-bearing rats. Results showed, with a high level of details, the effects of the MRT treatment on the nervous and vasculature tissues one-to-five months after the treatment (i.e. late-term radiotherapy effects). The correlation of PCI data with histologic results, on the same samples, was unable to determine the specific nature of the visualized lesions. In order to correctly classify and, lately, to understand the mechanisms leading to the radio-induced effects and especially the early stages of tissue ablations and Fe and Ca deposits formation, we need to treat new animals using the same MRT parameters already used in past experiments to study the produced lesions at earlier stages as recommended by expert neuropathologists.

The proposed experiment is one of the steps of a broad multi-annual study including a series of synchrotron radiotherapy and analysis experiments, part of which have been already realized; this proposal, and others that will follow, will allow reaching the statistical significance required for the study.

This experiment consisted of **two experimental sessions: the first one for the irradiation of the rat brains (April 2021) and the second one for PCI-microCT of the extracted and formalin fixed rat brains (June 2021).**

- **Scientific and experimental backgroud**

A preliminary PCI-microCT study (Barbone et al., Int J Radiat Oncol Biol Phys. 101,4, 2018) by our team put the basis for this experiment. In this previous work, different irradiation geometries (i.e. standard homogeneous–broad beam, MRT and millimetre-wide beams-called minibeams) on both healthy and glioblastoma-bearing rats were used and compare in terms of specific radiation-driven effects on the treated tissues (data from MD1037 and MD1121 experiments). It was possible to investigate the curing effect of the three geometries as tumour shrinkage and, among all, MRT gave the most interesting results. As shown in the figure 1, MRT treated rats showed the formation of hyperdense structures (Fe and Ca deposits) along the beam path and unspecific lesions recognized as tissue ablations. In this experiment, MD1219, we intended to access important new information on short-term healthy-tissue response after treatment to understand at a deeper level the early-stage damage caused by MRT on healthy tissues. We aimed at investigating at which delay after the irradiation Fe and Ca deposits are produced. This information is essential to check the hypothesis of the derivation of these microcalcifications from Fe deposits caused by vessel micro-bleeding. Correlation of PCI-microCT data with brain Hematoxylin & Eosin (H&E), Ca and Fe stained histology analysis is also planned.

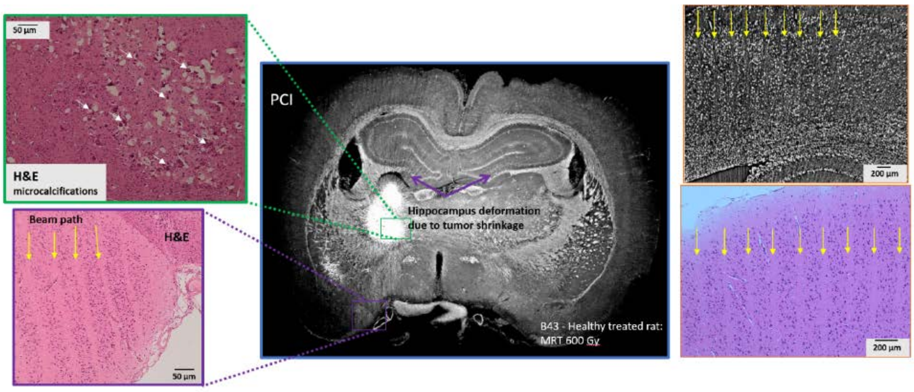

Figure 1

Based on the scientific and technical experience gained in the previous experiments, in MD1219 we irradiated healthy rats and sacrificed them at two different 'early' time points post irradiations to analised the short-term effets of the tissue. PCI-microCT was then applied to examine the treated and the control full rat brains in 3D and high sensitivity.

- **The experiment**

Animal irradiation session

32 male Fisher rats were anesthetized (5% isoflurane inhalation and ketamine, 64.5 mg/kg, + xylazine, 5.4 mg/kg, i.p. injection) and positioned on a stereotactic frame. Rat brains were then irradiated with unidirectional microbeams 50 µm wide and a center-to-center (c-t-c) intermicrobeam distance of 200 µm, resulting in an irradiation field of 5x8 mm^2 in the antero-posterior configuration. The irradiation field will be centered on the right-side brain using a well-established protocol, which includes the use of a 2D X-ray radiography of the rat skull. Radiation effects were explored **considering different groups of rats**, which were treated with the following MRT protocols:

Group 1: unidirectional MRT - 600 Gy (peak dose), 50 µm wide, 200 µm c-t-c:

Group 2: unidirectional MRT - 400 Gy, 50 µm wide, 200 µm c-t-c; n=

Group 3: unidirectional MRT - 200 Gy, 50 µm wide, 200 µm c-t-c;

Group 4: untreated animals – control group.

Two different time points were considered for each group: T1=14 days and T2=28 days, post irradiation.

Imaging session

<u>*Sample preparation for imaging*:</u> The day before their sacrifice, animals underwent an MRI at the GIN (Grenoble) for correlation with the X-ray based imaging analysis. After sacrifice, all brains were dissected out for imaging purposes, fixed in 4% formalin first and then, before the imaging session, put in an ethanol solution and stored in sealed plastic containers.

<u>*X-ray Phase contrast brain imaging set-up and experimental parameters:*</u> The propagation-based phase contrast microCT imaging set-up with a 3 µm optics coupled to the PCO.Edge 5.5 camera, a 35 keV monochromatic X-ray beam and a sample-to-detector distance of 170 cm were used to image the 32 samples. The imaging parameters were chosen to cover the full organs. For imaging the following beamline parameters were set: wiggler-w150 gap=54, wiggler-w125-gap=60, filters: Cv=0.8mm, Al=2.5mm).

- **Data analysis and preliminary results**

The CT reconstruction (with filtered back projection algorithm) and image processing of these data are in progress. Quantitative PCI image analysis will include image segmentation to derive a 3D visualization of the brain lesions, as in our work Romano et al. Cancers, 13, 2021, where we summarized the results of our previous experiments at the ESRF within this project.

The CT images reconstructed until now are of high quality and are almost artefacts-free. An example of an PCI-microCT image of a healthy rat brain irradiated in MRT with a peak dose of 600 Gy and sacrificed 28 days post irradiation (T2) is reported in figure 2. The tissue ablations caused by the microbeams are clearly depicted as well as the hydroxyapatite deposits starting to form along the paths of the microbeams.

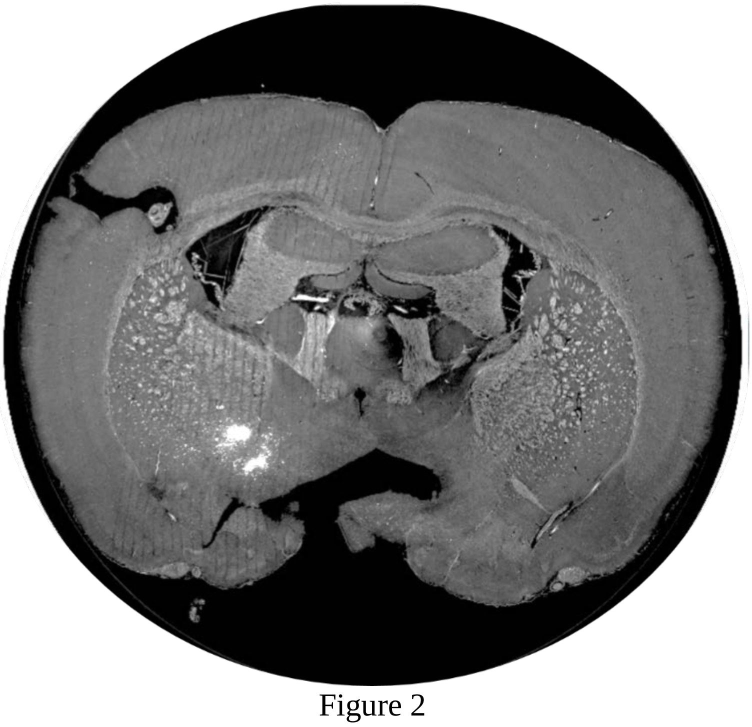

Figure 2

- **Concluding remarks and acknowledgements:**

This study is of paramount importance to complete the classification of radiation-driven effects induced by MRT and to channel the efforts towards a new X-ray based treatment for brain tumors.

This experiment, including both the animal irradiation and the imaging sessions, went off smoothly without major problems.

We are grateful for the help provided to us by the local contacts, the teams of ID17 and the ESRF Biomedical Facility. Technically and experimentally the beamtime was very successful.