EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Gaining first biological data for pencilbeam radiotherapy **---Final Report-- updated**	**Experiment number**: MD 533
Beamline: ID 17	**Date of experiment**: from: February 05 to: February 07, 2011	**Date of report**: Aug 22 , 2011
Shifts: 9	**Local contact(s)**: Elke Bräuer-Krisch	*Received at ESRF:*

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Report: This was our first experiment using the pencibeam radiotherapy technique recently developed at ID 17. The aim of the experiment was to develop the logistics of the technique for *in vivo* experiments and identify the limits set by both technical and biological mechanisms when pencilbeam radiotherapy is used for whole brain radiotherapy. The latter would be clinically releveant where a malignant primary brain tumour is extensively infiltrating or where multiple brain metastases are present.

Since the plan was to conduct both motor and behavioural testing after the radiotherapy, we worked in previously healthy adult male C57 BL/6J mice, since this is one of the best strains for behavioural studies.

Using a single stack MSC (EMSC, Ref.1) with an exposure and displacement method, this pencilbeam technique was used to irradiate the entire mouse brain within a period of 2/6 minutes for 400/200 ctc respectively with a new Krypton gas filter in place. Gafchromic Film (Nuclear Associates, NY, U.S.A.) was used to verify applied irradiation doses and irradiation patterns.

RESULTS:

For those experiments, we found:

1. The beam profiles obtained by Monte Carlo calculations are shown in Figure 1.

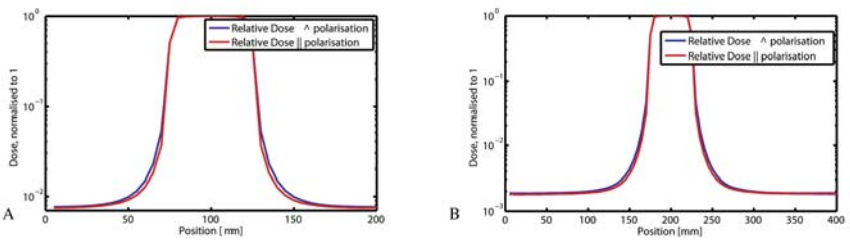

Figure 1: **Beam profiles for ctc 200 µm (A) and ctc 400 µm (B) at 3 mm depth**. Profiles parallel and perpendicular to the polarisation vector are shown.

2. The irradiation patterns for c-t.c 200 µm and 400 µm are shown on microscopy images of the lateral gafchromic films (Figures 2A+B); axial histology sections showed a good correlation with the pattern recorded on the anterior gafchromic film (Figures 2C+D). As microscopic lesions are best seen in cell-dense structures like the cerebellum, the pattern recorded on the lateral gafchromic films was reproduced well in the sagittal microscopy sections of the cerebellum (3 A-F).

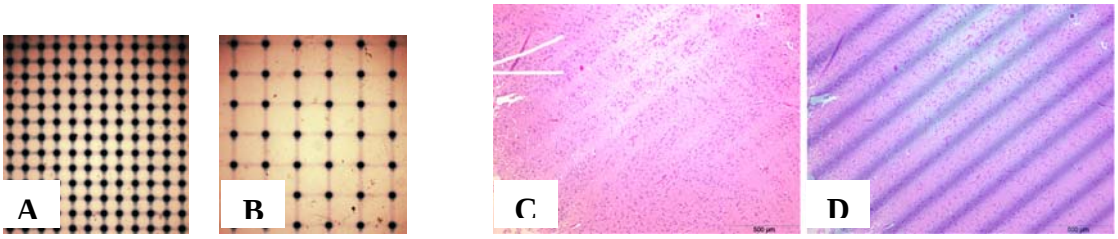

Figure 2: Microscopy image of the lateral gafchromic film after exposure with c-t-c 50-200-50 (A) and c-t-c 50-400-50 (B). Microscopy images of histology sections (H&E stain) showing almost no vital cells in the former path of the beam (C), correlating well with the irradiation pattern recorded on the anterior gafchromic film (D).

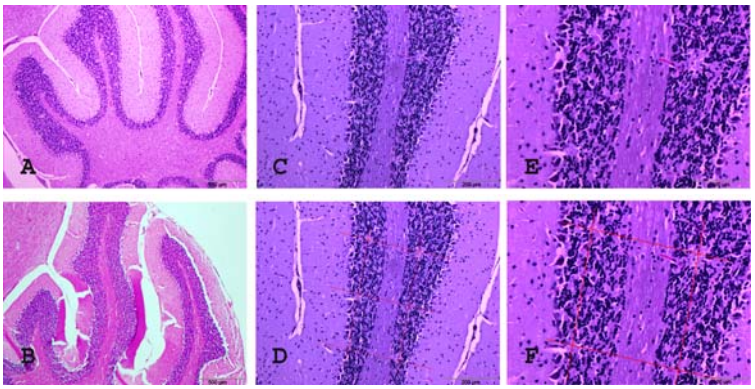

Figure 3: C57 BL/J6 mice, 6 months after irradiation with a peak dose of 172 Gy / valley dose of 1.72 Gy, ctc 200 µm (A, C-F are enlargements from sample A) and healthy control (B).

The lighter spots almost devoid of cells correspond to the ctc 200 µm lateral Gafchromic film profile as can be demonstrated by the overlaid patterns of histology section and distance grid with 200 µm lateral dimensions (D and F).

3. The measured peak-to-valley-dose ratio (PVDR) was about 100 for a c-t-c of 200 µm and about 400 for a c-t-c of 400µm. Those values were close to the PVDR expected by Monte Carlo calculations

4. With a c-t-c of 200 micron between the 50 micron FWHM spots, we reached the LD50 at a peak dose of around 1,164 Gy. No animals died with a peak dose of 862 Gy.

5. With a c-t-c of 400 micron between the 50 micron FWHM spots, no deaths occurred up to peak entrance doses of 2,298 Gy.

6. Testing of motor abilities on the rotarod showed impairment at one week after irradiation in all groups, compared to non-irradiated controls; at two weeks after irradiation, recovery was seen in all groups, some already being equal to the healthy animals again (Figure 3). The overall results were better for the animals with a 50-400-50 than with a 50-200-50 c-t-c. In all groups, including healthy animals, a deterioration of motor abilites was observed with age.

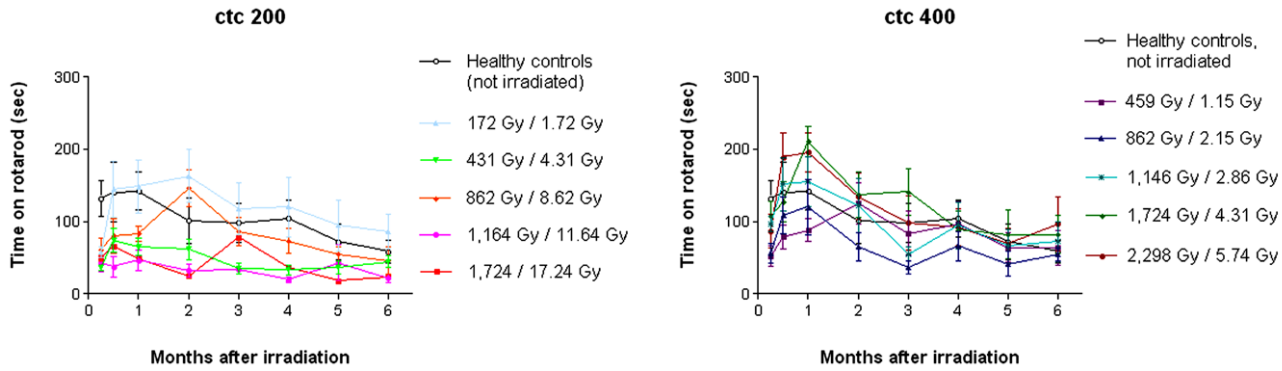

Figure 3: Results of the rotarod testing over 6 months (in brackets given the peak/ valley doses).

Performance was generally better in the c-t-c 400 µm groups with a PVDR of ~ 400 (B), compared to the animals in the c-t-c 200 µm groups with a PVDR of ~ 100 (A).

7. A memory loss was observed with age in the healthy group over the 6 months observation time. The ability to form new memory was generally better in the c-t-c 50-400-50 groups, compared to the 50-200-50 groups. At the end of the observation time (six months after irradiation) the recovery after temporary memory problems during the first month was such that with a c-t-c 50-400-50 the group averages were closer to that of the healthy animals then in most of the c-t-c 50-200-50 animals (Figure 4).

8. In determining the memory ability the most important factor seems to be the c-t-c ratio.With equal peak doses, the memory was better in the groups with higher c-t-c- ratio. Where the valley doses were equal, again the groups with a higher c-t-c ratio seem to be favoured with a better capability to form new memory content.

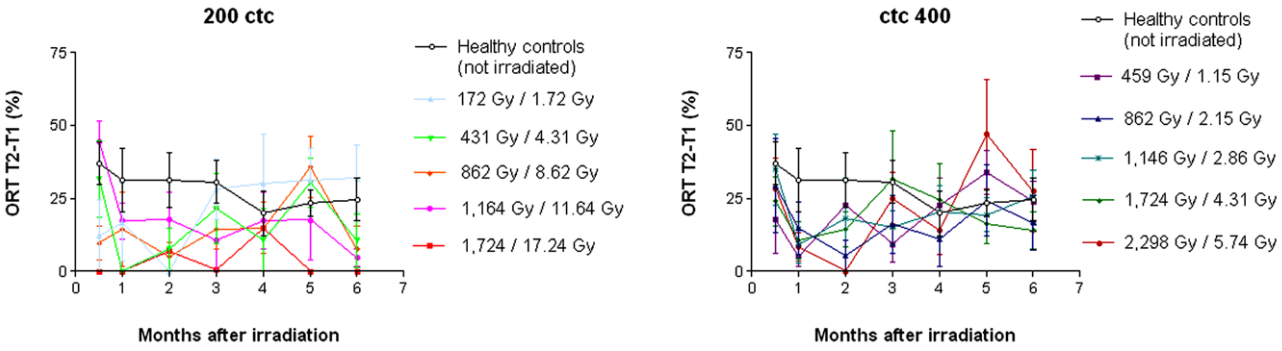

Figure 4: Results of the Object recognition test (ORT) during the 6 months observation time (in brackets given the peak/ valley doses).

Performance was generally better in the c-t-c 400 µm groups (B), compared to the animals in the c-t-c 200 µm groups (A). The initial drop of memory performance is lagging behind that of motor performance by several weeks.

We wish to thank everybody at ID 17 for helping to make our experiments running as smoothly as they did. Our special thanks go to Michel Renier, who came up with the idea of pencilbeam radiotherapy in the first place and developed special hardware, and to our local contact, Elke Bräuer-Krisch, for her energetic and knowledgable support before, during and after our experiments and for thinking of and alternative approach to conduct pencibeam radiotherapy in higher dose ranges. We also thank Yolanda Prezado for conduction preliminary calculations on which we could base the final planning of our experiments. We thank Stefan Bartzsch for the final Monte Carlo calculations. We thank Herwig Requardt, Christian Nemoz and Thiery Brochard for the 'Behind the scenes' preparation before we started. Dominique Dallery is thanked for all preparation of the animal experiments and the expert care of our animals, together with Charlene Caloud.

1) E. Bräuer-Krisch, H. Requardt, T. Brochard, G. Berruyer, M. Renier, J. Laissue and A. Bravin (2009) New technology enables precision multi slits collimators for MRT (Microbeam Radiation Therapy), *Rev Scientific Instr* 80(7):074301.