EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Evaluating microbeam radiation therapy (MRT) for malignant brain tumors and vascular malformations: an animal experimental study.	**Experiment number**: MD 131-3
Beamline: ID 17	**Date of experiment**: from: April 26 to: May 1, 2006	**Date of report**: Sep. 9, 2006
Shifts: 15	**Local contact(s)**: Elke Bräuer-Krisch	*Received at ESRF:*

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Report:

These experiments were in part repeats of the experiments conducted during MD 131-1 in spring 2005, to increase the number of animals in the experimental groups using C6 glioma in Wistar rats. We also introduced a new experimental set, administering 5 mmol / kg glutamine 2 hrs prior to and 2 hr after MRT. MRT was performed two weeks after implantation of 100,000 C6 glioma cells, in bidirectional mode, with the beam arrays intersecting in an orthogonal fashion at the site of the primary tumour. With 350 Gy per unidirectional irradiation, the dose deposited in the tumour was about 700 Gy. We used 10 by 14 mm arrays of 50 quasiplanar microbeams, with a beam width of 24.75 micrometer and a centre-to-centre distance between the microbeams of 211 micrometer, generated by the TECOMET collimator at ID 17. A total of 125 animals were used for these experiments.

We were hoping to use the Frelon camera to verify the presence of a tumour in each animal before MRT. The idea was to systemically inject iodinated contrast agent and acuire a set of images for K-edge digital subtraction. However, despite extended efforts by the ID 17 colleagues we were unable to generate meaningful images that would have allowed us to verify the presence of tumour in our animals. Besides glitches in the computer software that made it difficult to acquire equally-sized images above and below the iodine K-edge as requirement for K-edge subtraction angiography, the technique in the present set-up might not be sensitive enough for the minute amounts of contrast agent accumulating in the very small tumours. A possible option to try verifying tumours before MRT in the same hutch might be CT.

The survival results of the repeat experiments matches well the results from the first set of experiments in spring 2005. Interesting results were obtained by introducing a new analysis method, called object recogntion test (ORT), which is a cognitive test deigned to evaluate memory formation. The test is based on the natural curiosity of rodents and the resultant tendency to explore objects within their reach. This test has originally been described by Ennaceur and Delacour (Enanceur and Delacour, 1988), modified by Ennaceur and colleagues (Ennaceur et al, 2005) and in other laboratories (Yatsiv et al, 2005). Two tests (T1 and T2) are conducted. The duration of each of those tests is 4 minutes. During the first test (T1), the animal is presented with two identical objects fixed to the floor of the glass cage. The animal will spend about equal time periods exploring each of these objects. For the second test (T2), one of the previously presented objects is exchanged for a new object. During both tests, the time that the animal spends exploring each object is registered with two stopwatches by a researcher blinded to the treatment. The ORT is based on the assumption that an animal with proper new memory formation will remember the first object from the earlier exploration and now spend significantly more time exploring the new object than re-exploring the object previously encountered. If memory formation is not optimal, the animal would not remember the previously encountered object and more equal time periods would be spend on exploration of both the previously encountered and the newly introduced objects. For purposes of statistical analysis, the registered absolute time values are converted into percent of object exploration time and the time differences between exploration time spend more on the on the new as compared to the previously encountered object (ΔT2) are plotted. Advantages of this test are that no prior training of the animals is required but rather the natural curiosity of the animals is exploited and the interference of the experimenter is minimal. Since decline of cognitive function is a well-known adverse effect of brain irradiation, we believe that the ORT can give us valuable information about possible adverse effects of MRT. Based on results from the ORT, we will be able to adjust treatment protocols before proposing clinical trials.

First, we verified that similar to what is known from human patients, the presence of a malignant tumour severely decreases cognitive function (Figure 1).

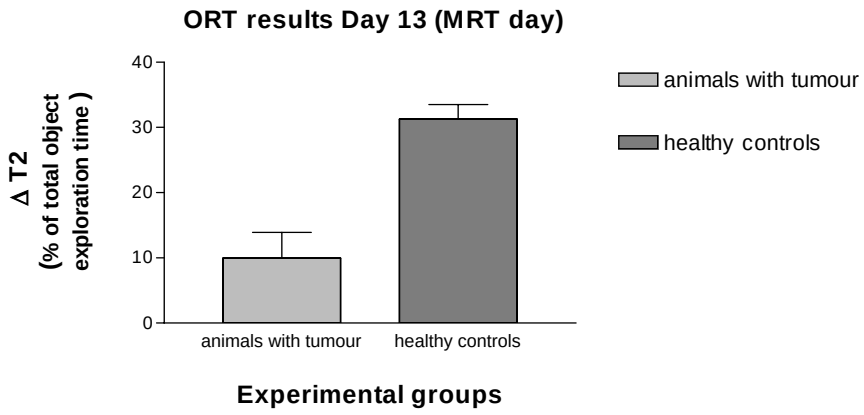

Figure 1: Results of the ORT on day 13 after implantation of 100,000 C6 glioma cells (equals MRT day). There is a significant memory deficit observed in animals with tumours, as copared to healthy animals (n = 7).

Next we observed that animals that had received BSO injections into the tumour in order to render the tumour more radiosensitive before MRT (an effect that was definitely achieved), unfortunaley suffered a significant memory loss early after BSO injection / MRT.

This was true for both tumour bearing animals and healthy animals which were given BSO injections into a brain location corresponding to the site of the tumour (Figure 2). We believe that, in the tumour-bearing animals, this memory loss might be a consequence of the presence of the malignant tumour and a leakage of BSO into the healthy brain tissue. Given our previous enthusiasm regarding BSO as a radiosensitizer, this was rather disappointing. Memory formation improved in the animals injected with BSO when tested at 2 and 3 months after MRT.

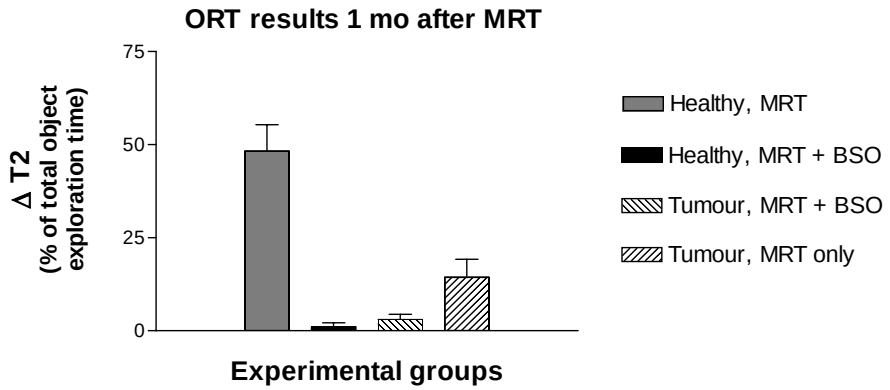

Figure 2: Results of the ORT 1 month after MRT and BSO injection.

However, we were pleased to observe that injection of glutamin prior to and after MRT seems to protect memory formation (Figure 3).

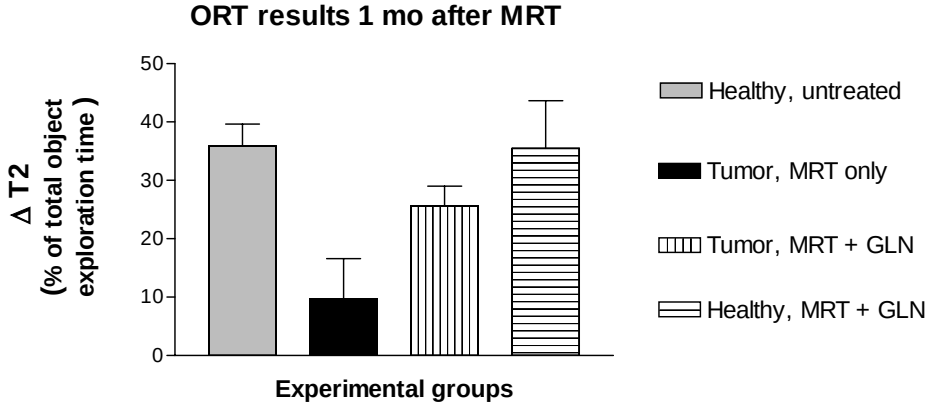

Figure 3: Results of the ORT 1 months after MRT and glutamine injection.
Glutamine has definitely a memory protective effect in this experiment (n = 9).

As a conclusion from our experiments, we now consider combining BSO and glutamine injections, in order to keep the radiosensitizing effects of BSO yet to protect memory function.

We wish to thank Mr. Dominique Dallery for his excellent support in the animal facility, Ms. Catherine Massart for her support in the cell culture facility, Drs. Herwig Requard, Thierry Brochard and Christian Nemoz for their efforts to set up imaging in the MRT hutch. Once again, all embers of our research team felt well supported and taken care of by the friendly reception at the ESRF.