EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** **Radiotherapy of absence epilepsy: effect of microbeam irradiation on thalamocortical pathological oscillations**	**Experiment number**: MD531
Beamline:	**Date of experiment**: from: Sept 11th, 2010 to: October 27th, 2010	**Date of report**: 17/09/2012
Shifts: 2x12	**Local contact(s)**: Christian Nemoz and Elke Brauer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Rationale:

MD438 beamtime allocation allowed us to investigate the effects of synchrotron-generated interlaced MRT coupled to pink-beam imaging (Serduc et al., 2010b) on a well-validated rat model of absence epilepsy, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS). Since recent data had shown that absence seizures are initiated within a «cortical focus» - the somatosensory (S1) cortex - before their diffusion to the rest of the cortex and the ventro-basal thalamus (Polack et al., 2007; David et al., 2008), we irradiated this cortical region bilaterally using 4 ports of 50µm-wide microbeam resulting in 200Gy delivery into the targeted areas. This procedure resulted in 50% reduction of the cumulated seizure duration compared to sham animals. This effect was significant as soon as 3 week after irradiation, and still constant after three months. As opposed to cortical irradiation, no beneficial effect was observed when the thalamus relay nuclei – also involved in the network underlying absence seizures – was bilaterally targeted using the same parameters.

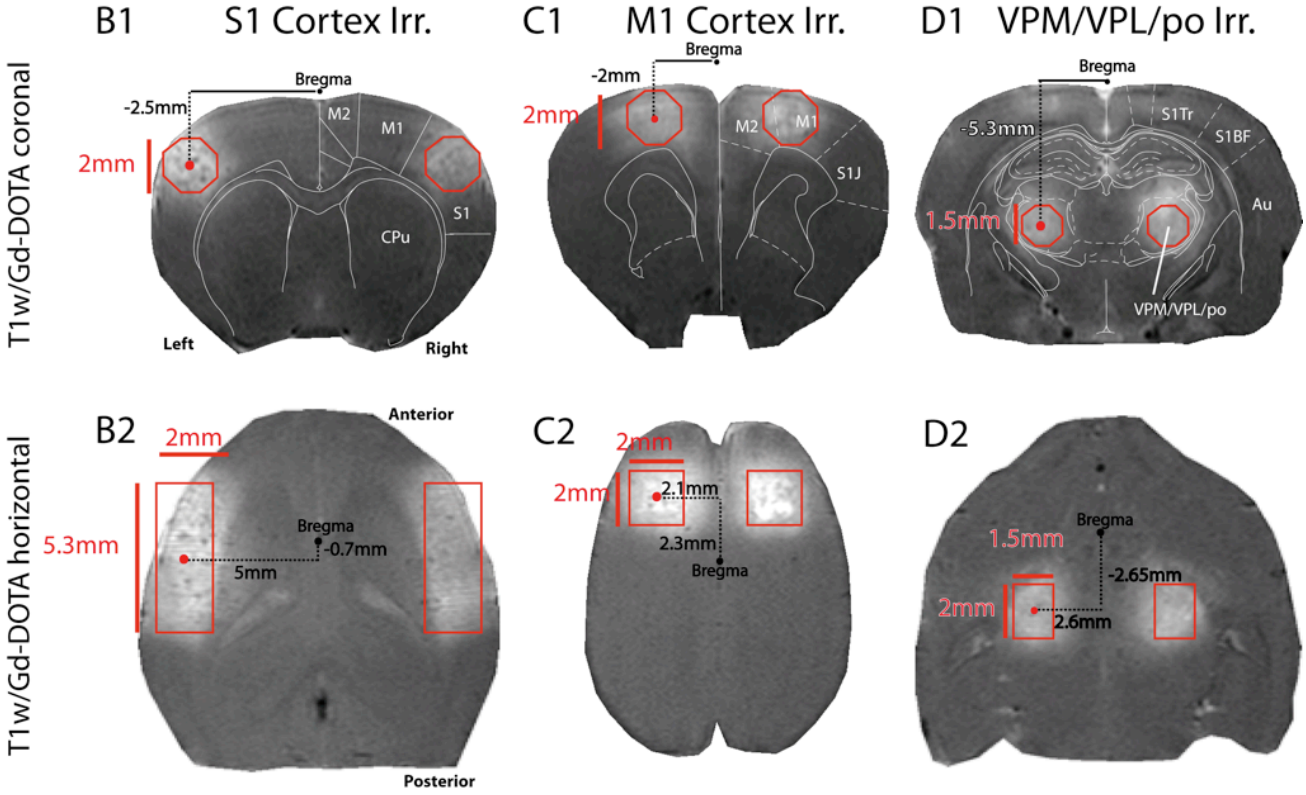
As of today, it is unknown why irradiation of the cortical focus did not totally abolish absence seizures. The partial effect of the cortical irradiation raises the question of whether irradiated structures retain some epileptogenic properties. We propose to answer this question using intracellular recordings of neurons within the irradiated cortex in order to demonstrate

whether the irradiated cells still display the characteristic hyperexcitability of the GAERS' neurons. These recording will be performed in collaboration with Stéphane Charpier (Centre de recherche du cerveau et de la moelle, CRICM, Paris). This team, with which we have been successfully collaborating in the past (Polack et al., 2007), is very familiar with the GAERS model and the functional characteristics of its cortical and thalamic neurons.

Methods:
Using the IntMRT technique described in Serduc et al. (2010), we targeted bilaterally the so-called "cortical focus" (200Gy), i.e. the somatosensory cortex (S1Cx), as well as two structures involved in the propagation and maintenance of SWDs, i.e. the motor cortex (MoCx) and the ventro-lateral thalamic nuclei (VLTN; Fig. 1). In each animal, we verified the correct targeting by T1-weighed magnetic resonance within the two weeks that followed IntMRT and examined their general behavior by standard tests to detect possible side effects. During the 4 months following irradiation, we monitored weekly the seizures in freely moving animals by recording local-field potentials (LFPs) from depth electrodes implanted in the three target regions. Finally, we investigated the mechanisms underlying the antiepileptic effect by performing *in vivo* intracellular recordings of individual irradiated neurons of the S1Cx.

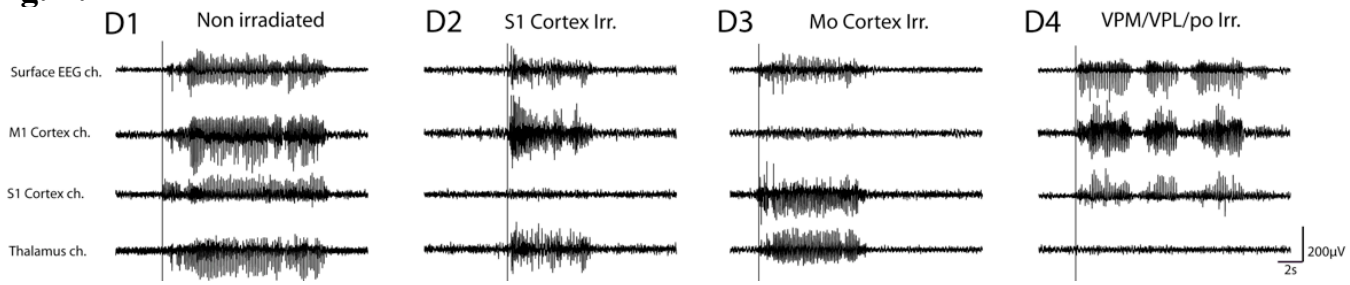
Results:
MRI acquired 14 days after bilateral S1Cx, MoCx or VLTN (Fig 1) irradiations showed strong, sharply delimited, hypersignals in the regions where MBs interlaced, suggesting an alteration of the vascular integrity and micro-ruptures of the blood-brain barrier within the targets. These images denote a precise targeting and an effective sparing of tissue irradiated with non-interlaced MBs.

Fig. 1:



The bilateral delivery of 200 Gy in any of the three brain targets resulted in a near complete abolition of SWDs on the LFPs recorded from these structures (Fig 2). The average power in the 6-9 Hz frequency band was decreased by 93, 74 and 88% in the irradiated S1Cx, MoCx and VLTN, respectively, when compared to LFP signals recorded from homologous structures in non-irradiated animals (Fig 2).

Fig. 2:



In vivo intracellular recordings showed that irradiated pyramidal neurons were strongly hyperpolarized, displayed a decreased excitability and a reduction of spontaneous synaptic activities (Fig. 3). As a result, irradiated cortical neurons were unable to oscillate in synchrony to initiate spike-wave discharges (Fig 4).

Fig 3:

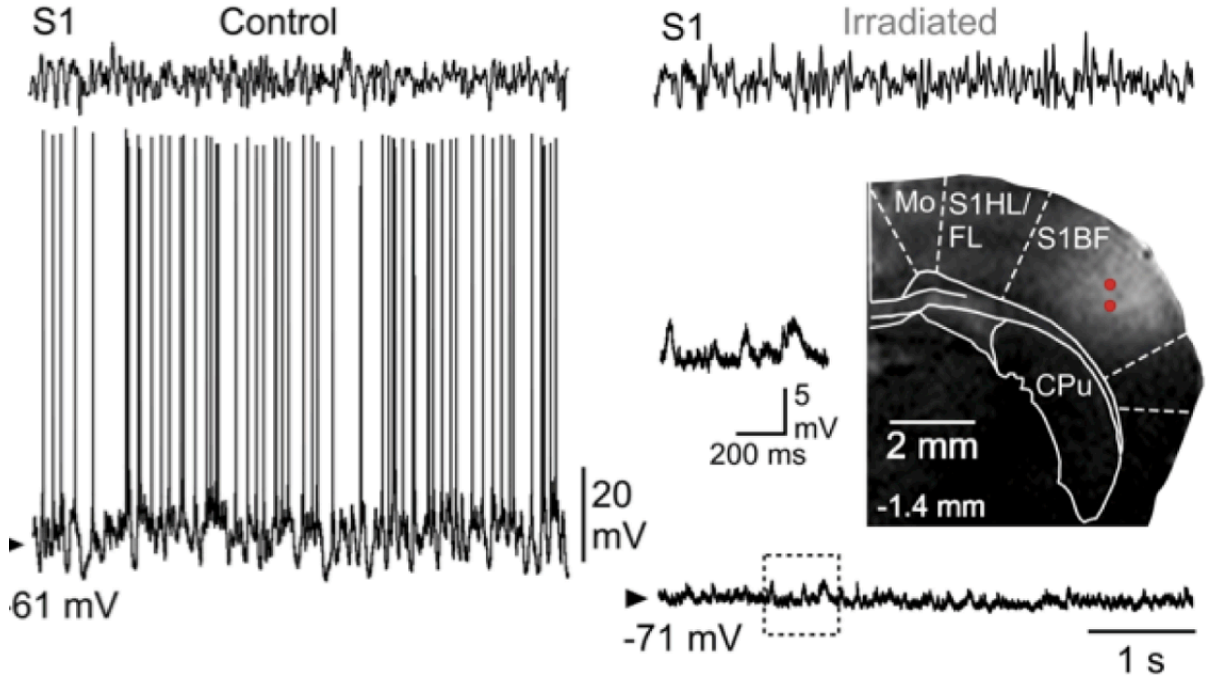
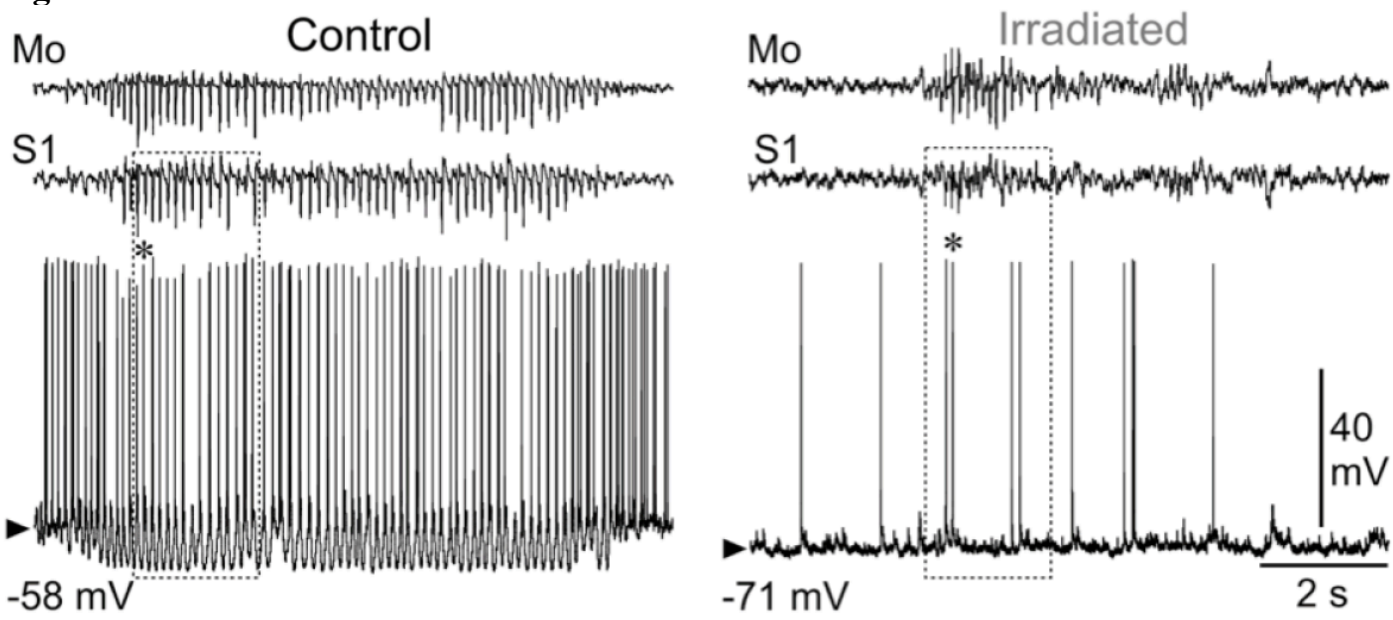


Fig. 4:



Conclusions:

These above-mentioned functional alterations explain the suppression of large-scale synchronization within irradiated cortical networks. However, the fact that seizures remained in the non-irradiated structures of the thalamocortical loop (see Fig. 2) indicates that the destruction of the cortical focus is not sufficient to totally abolish pathological oscllations. We propose that other structures can indeed start seizures but with a lesser efficiency.

Valorisation:

- **Written material:**
  Pouyatos et al. - Synchrotron x-ray interlaced microbeams suppress paroxysmal oscillations in neuronal networks initiating generalized epilepsy. Submitted.
  Pouyatos B (2010) Cartographie et radiochirurgie du foyer des décharges de pointes-ondes dans un modèle d'épilepsie absence. 13èmes journées françaises de l'épilepsie. Grenoble 11-13 Novembre 2010. Epilepsies. 22-Suppl.1.22:23.

- **Communications:**
  ANT neuromeeting Jan. 2010 (Lecture)
  Journée d'animation des neurosciences de Grenoble, May 2010 (Lecture).
  France 5 – Le journal de la santé (TV program) Juin 2011.