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:** **Synchrotron-generated X-ray interlaced microbeam therapy of mesiotemporal lobe epilepsy**	**Experiment number**: MD571
Beamline: 17	**Date of experiment**: from: July 6th, 2010 to: July 8th, 2010	**Date of report**: 19/09/2012
Shifts: 6	**Local contact(s)**: Christian Nemoz and Elke Brauer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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François Estève - Grenoble Institut des Neurosciences, INSERM U836

Report:

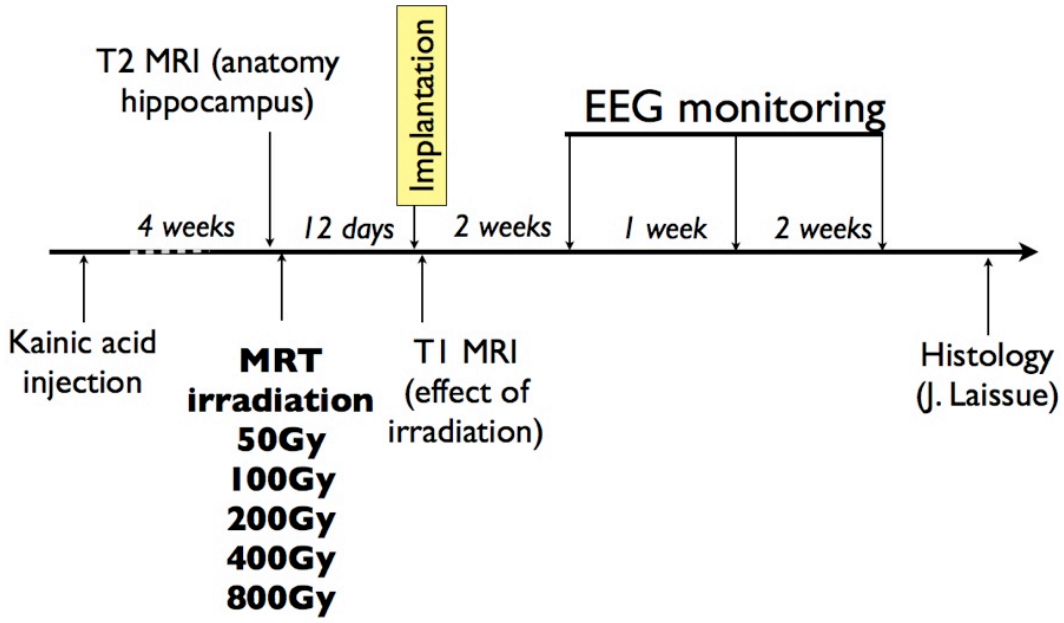
Rationale:

MD438 and MD531 beamtime allocations allowed us to investigate the effects of synchrotron-generated interlaced MRT coupled to pink-beam imaging on the GAERS rat. Since recent data had shown that absence seizures arise within the somatosensory cortex before they diffuse to the rest of the cortex and the thalamus, we irradiated this cortical region bilaterally using 4 interlaced ports of 50µm-wide microbeams resulting in a homogenous dose of 200Gy into the targeted areas. This procedure resulted in a 50% reduction of the seizure duration compared to sham animals that appeared as soon as 3 weeks after irradiation and was still significant after five months. Histological and electrophysiological data suggest that this effect is not mediated through simple tissue necrosis, but probably via a disruption of the local cellular network, which prevents the neurons from hypersynchronizing and generating epileptic discharges, without impairing their ability to perform their physiological tasks. This non-destructive effect of MRT becomes extremely suitable when seizures originate from a brain structure with a critical role like the hippocampus - the structure most commonly involved in pharmacoresistant MTLE. ***In order to address the applicability of MRT on MTLE, we propose to investigate its effects on a predictive and drug-resistant model obtained after intrahippocampal injection of kainate in the mouse***. This mouse model was preferred to the one developed in the rat because of the stability and recurrence of the

seizures over a long time period, (ii) the resistance to several anti-epileptic drugs and because (iii) intracellular recordings were recently collected by our group in this model.

Methods:

- *Timeline*



- *Experimental groups:*
  - Irradiated kainate mice 50Gy n=5
  - Irradiated kainate mice 100Gy n=5
  - Irradiated kainate mice 200Gy n=4
  - Irradiated kainate mice 400Gy n=2
  - Irradiated kainate mice 800Gy n=1
  - Control kainate mice 0Gy n=6

Results:

- *Kainate injection*

  One of the two cannulas used for intra-hippocampal injections of kainate was occluded. As a result some of the mice did not become epileptic.

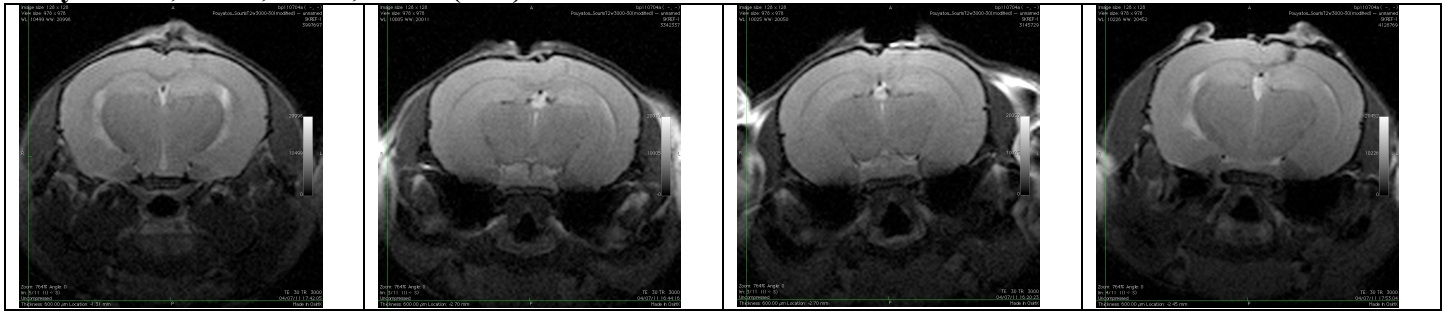
  The mossy fiber sprouting induced by the kainate injection was visible on the T2 anatomical MRIs performed before irradiations. All mice showing no hippocampal sclerosis were excluded from the results.

- *T2 anatomical MRI*

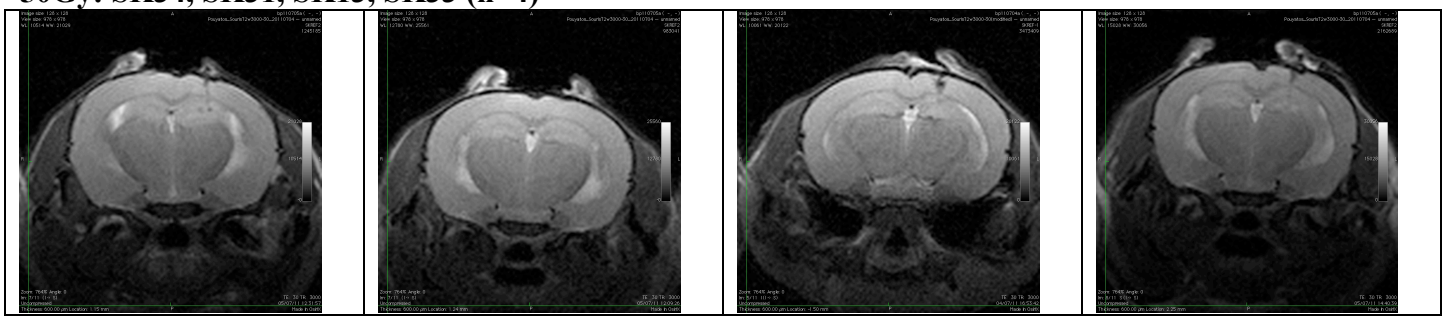
>>> Mice which indeed received kainate in their hippocampus show a clear hypersignal within the right hippocampus. Right hippocampus appears bigger, as a result of the sclerosis.

List of mice with sclerosis:

0Gy: SK17, SK15, SK28, SK16 (n=4)

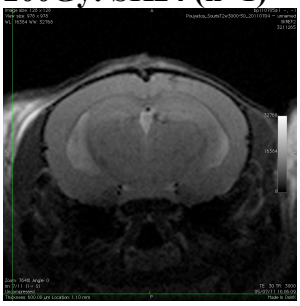


50Gy: SK34, SK31, SK13, SK33 (n=4)



100Gy: SK12, SK20, SK14, SK19, SK25 (n=5)

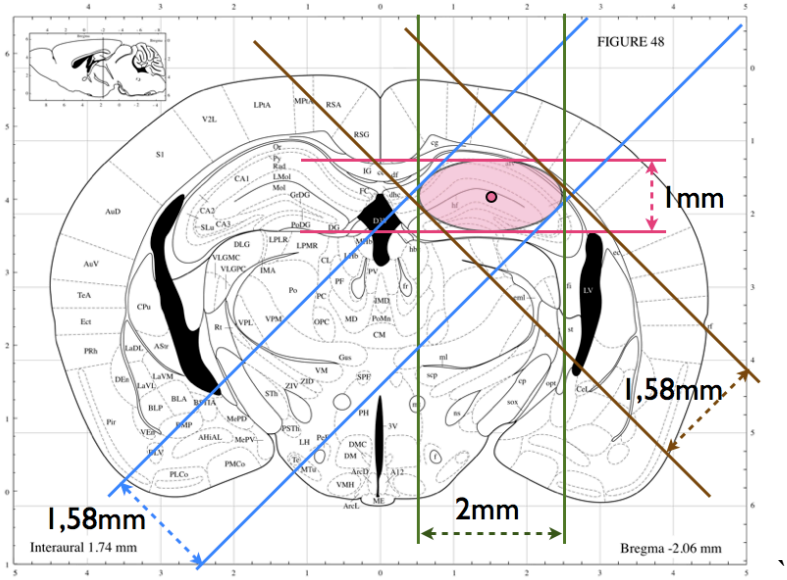
200Gy: SK24 (n=1)



400Gy: (n=0)

800Gy: (n=0)

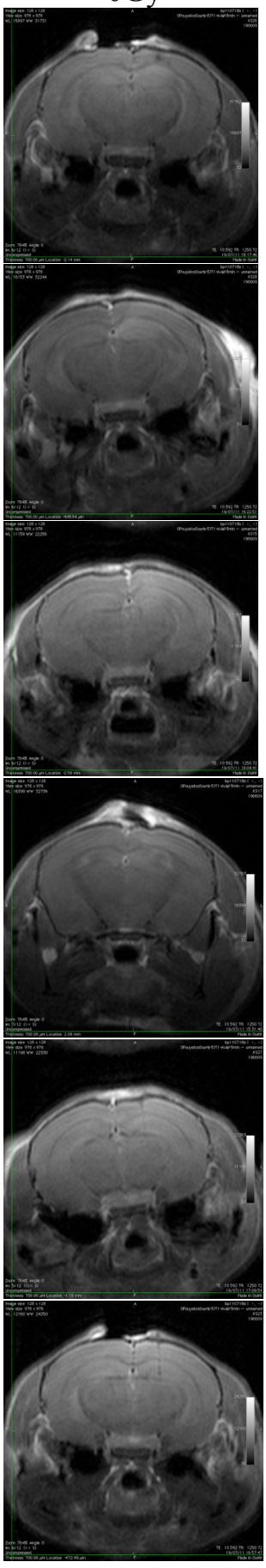
- Irradiation: we modified the size of the microbeam for the different ports in order to better fit the shape of the dorsal hippocampus.



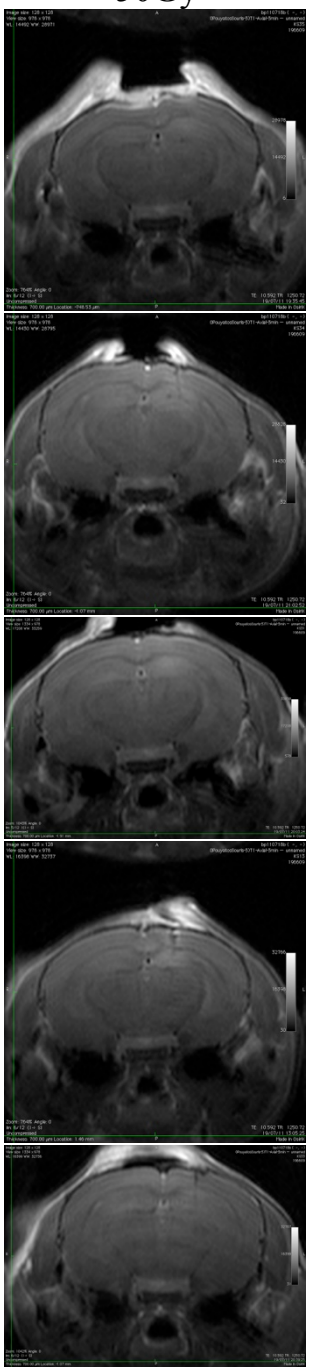
However, the posterio-ventral hippocampus (which did not receive the kainate injection) was not irradiated.

- 12 days post-irradiationT1-gado MRI

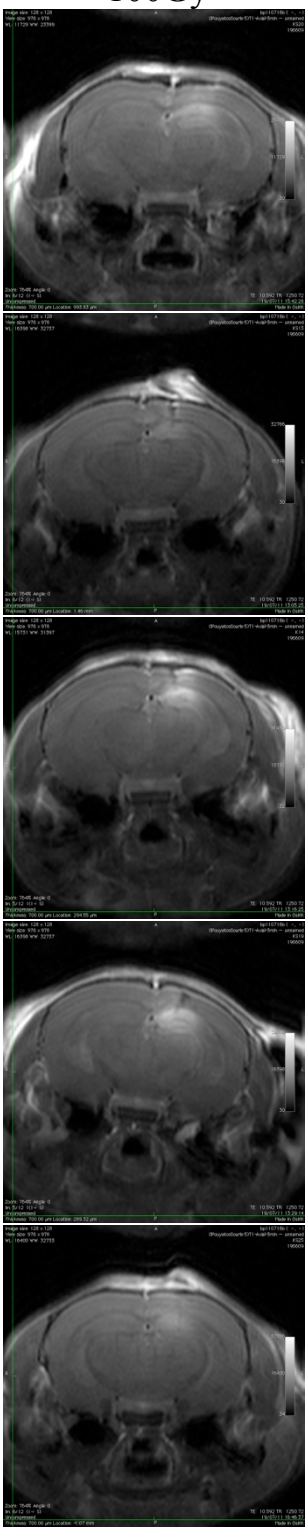
0Gy



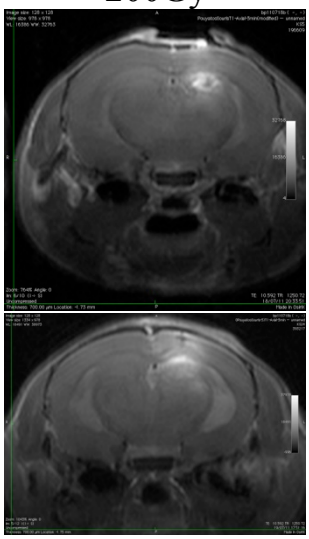
50Gy



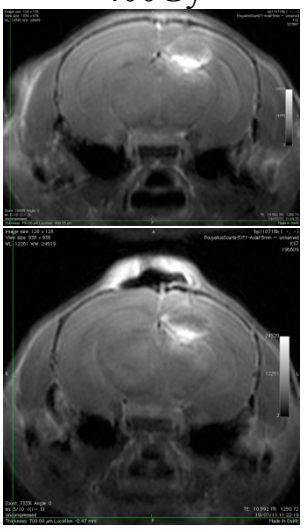
100Gy



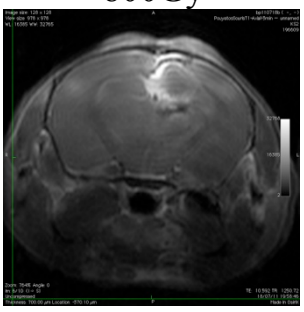
200Gy



400Gy



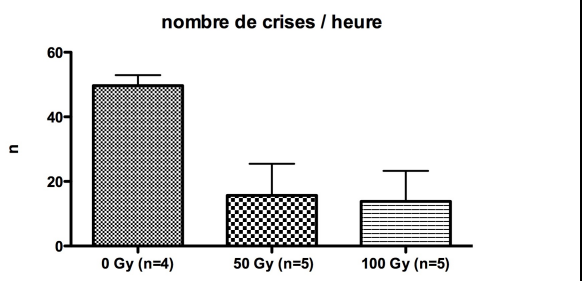
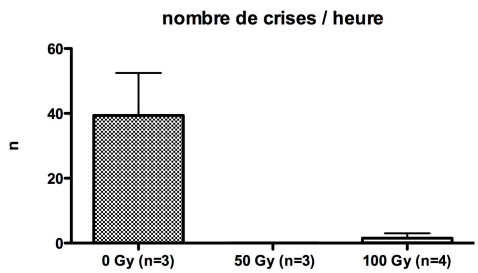
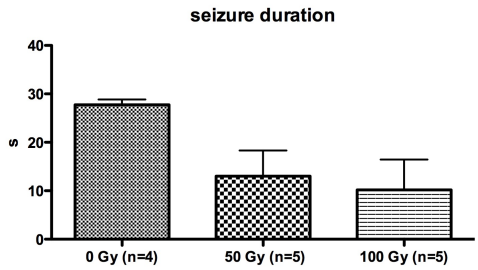
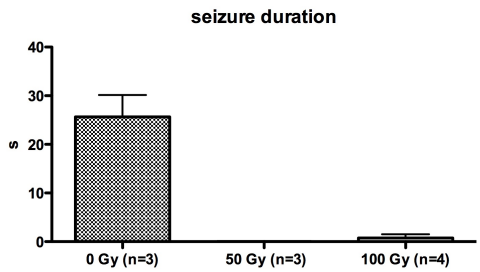
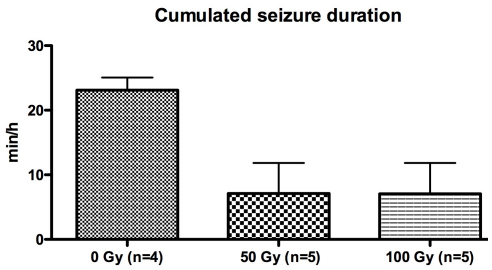
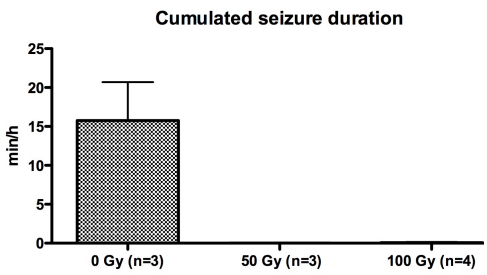
800Gy



>>> Hypersignals are related to gadolinium diffusion through vessel microruptures due to irradiation. Threshold of vessel impairment appears to be between 50 and 100Gy.

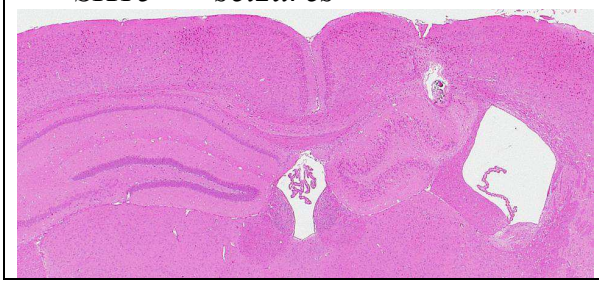
Note that 2 animals irradiated at 800Gy died within 1h post-irradiation (not shown here).

- *EEG*
  Are included only the animals which showed a hippocampal sclerosis on the T2-MRI and the histology.
  Note that some animal lost their headstage connector between 2w and 4w post-irradiation which explains the difference in the number of animals.

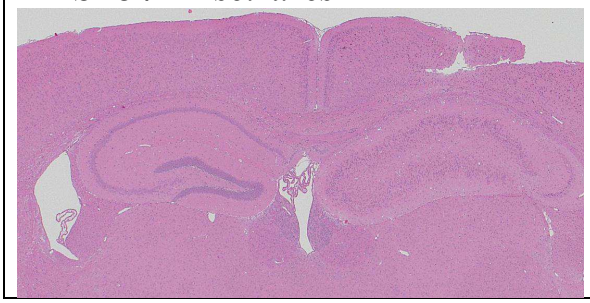
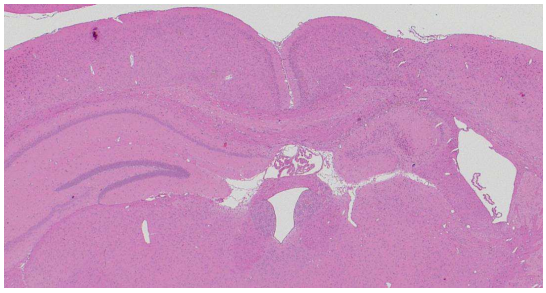
2 weeks post irradiation	*4w post-irradiation*
	
	
	

- *Histology:*
  0Gy:

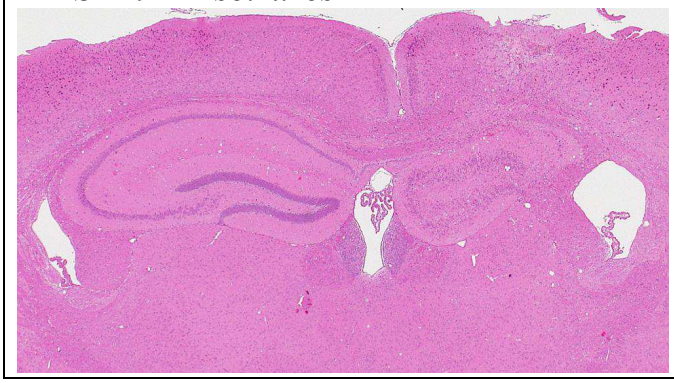
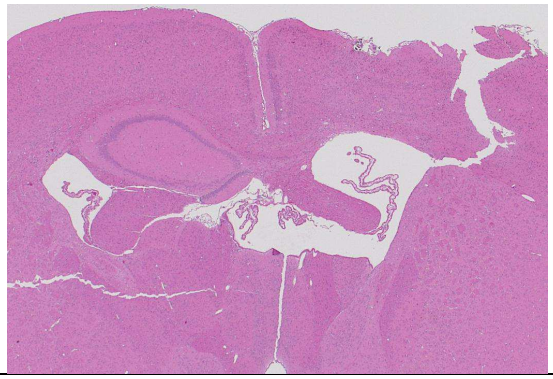
SK15 >>seizures



50Gy:

SK34 >>seizures	*SK13 >> No seizures*
	

100Gy:

SK19 >>seizures	*SK12 >> No seizures*
	

Conclusions:

- An antiepileptic effect was obtained at 50Gy and above.
- At 50Gy no necrosis was visible on histlogical slices. However, a functional effect was quantifiable. Neuromodulation?
- Despite the improvement in the conformation of the irradiation target, it is still difficult to target both the dorsal and the ventral hippocapus.
- The antiepileptic effect appears to increase between 2 and 4w post irradiation.