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 via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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: Anatomy of Central Nervous System Immune Privilege**	**Experiment number**: MD-1230
Beamline: ID17	**Date of experiment**: from: 12 Oct 2021 to: 13 Oct 2021	**Date of report**: 14 Jan 2022
Shifts: 3	**Local contact(s)**: Luca Fardin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Three experimental sessions were scheduled for this experiment for use of the beam:

- Experimental Session 1 (10-12 Dec 2020): Remote access post-mortem imaging
- **Experimental Session 2 (12-13 Oct 2021): Pilot in vivo experiment**
- Experimental Session 3 (18-20 Nov 2021): Full in vivo experiment

Experimental Session 2 (12-13 Oct 2021): Pilot in vivo experiment

Note: SMIS database notes this experimental session as encompassing 6 shifts. This is incorrect, as the session was scheduled from 12 Oct 8:00 to 13 Oct 8:00. Only 3 shifts were assigned.

In this experimental session, the cerebrospinal fluid (CSF) spaces in the mouse brain were imaged for the first time *in vivo*. Mice were injected with barium nanoparticle-based blood pool contrast agent (ExiTron nano12000) into the ventricle, the combination showing the best results in the post-mortem scans of the previous experimental session. Different injection volumes and injection rates were evaluated in terms of animal tolerance *in vivo*, achievable contrast and distribution throughout the CSF spaces.

Anaesthetic tolerance, length of procedure and proper management of body heat are further critical parameters for the survivability of animals. Therefore, before the official beamtime start, the user personnel familiarized themselves with the animal facilities and beamline hutches for optimal workflow, minimizing the time

between animal anaesthetic induction and start of the scan. In addition, a parallelized schedule between surgeries and scanning needed to be established to minimize waiting time between scans.

Mice were mounted in a revised version of the user-designed, mouse holder for *in vivo* skull SRµCT measurements (Fig. 1A). In order to increase the workflow speed, tooth and ear bars were redesigned to be continuously adjustable. A nose cone was added to increase the points of contact for improved mechanical fixation. The bars and the head piece as a whole were furthermore designed to be removable, allowing for quick replacement and continuation of the experiment in the case of a broken part.

Cardiac and respiratory cycles were monitored via electrocardiogram to enable retrospective gating. Due to cabling of the electocardiogram, breathing sensor and temperature probe as well as tubing of the water pump for maintaining body heat, only 180° rotation scans were possible with the setup. 2000, 30 000 or 60 000 projections were recorded per scan with 5 ms exposure time at 6.3 µm voxel size, using a 2560 pixel wide pco.edge 5.5 camera and monochromatic beam at a photon energy above the K-edge of barium (37 keV).

Injection of 5 µl of barium nanoparticles at 0.5 µl/min showed the best result in terms of contrast in the CSF spaces (Fig. 1B and 1C). The anatomy of CSF-spaces showed considerable difference in morphology comparing to the post-mortem images acquired in Experimental Session 1, supporting the need for *in vivo* imaging to capture anatomically correct data. Part of these differences can, however, also be attributed to the additional volume of fluid injected, as the injection rate is considerably higher than the natural production rate of CSF at 0.1 µl/min (Liu et al., 2020). As slower injection rates would exceed the available time under anaesthesia, however, continuous infusion of contrast agent during the scan would be required to achieve the necessary volume administered for sufficient contrast.

Datasets were reconstructed using either all projections, or using the recorded electrocardiogram for retrospective cardiac gating. Between 1500 to 4500 projections out of 60 000 could be used per stage in the cycle, depending on heart rate. Comparison between these two modes showed increased blurring of CSF spaces in the non-gated reconstructions, indicating blood flow-induced motion and confirming the need for gated reconstruction.

Scans with 60 000 projections showed larger motion artifacts compared to scans with 30 000 projections, regardless of gating, indicating non-periodic motion of the animal over the course of a scan. 360° scans would, therefore, be preferred in this type of experiment, as it would allow reconstruction of a 180° scan with half the projections without requiring an additional scan should similar drifts reoccur or only part of the scan be useable due to premature death of the animal.

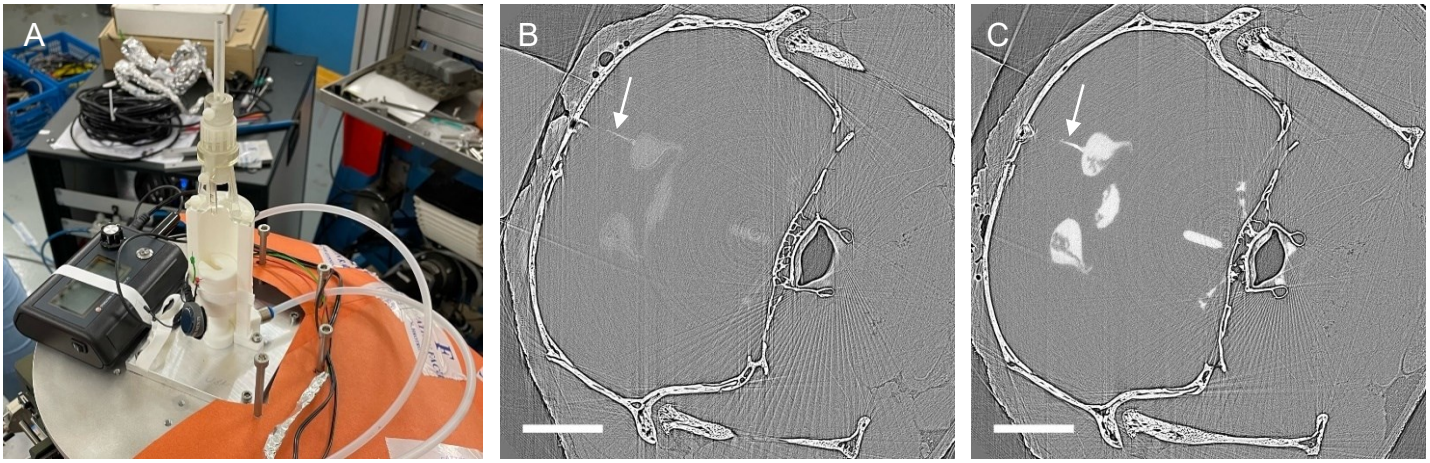

Fig. 1A: Revised version of the mouse holder installed on the rotation stage. Electrocardiogram cabling, temperature probe, breathing sensor and tubing for water pump-based heating are all visible. B: Virtual SRµCT slice displaying the contrast agent injection site into the lateral ventricles (arrow). 2.5 µl of barium nanoparticles were injected at an injection rate of 0.5 µl/min. C: Corresponding section of a mouse injected with 5 µl of barium nanoparticles at 0.5 µl/min. Scale bars: 2 mm.