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: 18 Dec 2021 to: 20 Dec 2021	**Date of report**: 14 Jan 2021
Shifts: 6	**Local contact(s)**: Luca Fardin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

University of Basel Biomaterials Science Center - BMC Gewerbestrasse 14 CH - 4123 ALLSCHWIL

Prof. Bert Müller

Dr. Georg Schulz

Dr. Griffin Rodgers *

University of Zurich Institute of Physiology The Interface Group Winterthurerstrasse 190 CH - 8057 ZUERICH

Prof. Vartan Kurtcuoglu

Dr. Britta Bausch *

Dr. Willy Kuo *

Marta Girona Alarcón *

ESRF 71 avenue des Martyrs CS 40220 FR - 38043 GRENOBLE Cedex 9

Dr. Alberto Bravin

Preliminary 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 3 (18-20 Nov 2021): Full in vivo experiment – *Preliminary Report*

During this session, the contrast agent and *in vivo* imaging protocol established in Experimental Session 2 was applied to a larger number of animals to derive anatomical parameters of mouse cerebrospinal fluid (CSF) space *in vivo*. Cabling for electrocardiogram, breathing sensor, thermometer as well as tubing for body heater and infusion pump were managed using a drag chain, allowing for full 360° rotation of the setup.

Mouse body temperature was monitored by a dedicated person outside the hutch during the animal mounting process, as large changes in positioning, heating element contact and heat insulation occur during the procedure. This allowed for minimal delays in temperature adjustment, leading to much more tightly controlled body temperature and greatly increased survival rate of the animals compared to the pilot study.

SRμCT images were acquired using monochromatic beam at a photon energy slightly above the K-edge of the chosen X-ray contrast agent (37 keV for Ba) and with an effective pixel size of 6.2 μm for a full-width scan of

the skull. A short exposure time of 5 ms was chosen to minimize motion artifacts and to capture sufficient projections per cardiac or respiratory cycle. An initial short fly scan with 2000 projections was acquired and reconstructed quickly to confirm positioning of the field of view, proper mounting and health status of the animal. Additional anaesthetics were administered if an elevation in heart rate was observed on the electrocardiogram. For the full scan, 60 000 projections with a full 360° rotation were then acquired while simultaneously recording cardiac and respiratory cycles for retrospective gating.

Mice were injected into the ventricle with 5 µl barium nanoparticle-based X-ray contrast agent (ExiTron nano 12000) at 0.5 µl/min. This experiment was repeated until fully complete scans of three male and three female live mice were acquired, which required a total of 10 animals.

In order to improve the physiological accuracy of the data and avoid artificially increasing the intracranial pressure, slower contrast agent injection rates would be required. As injection prior to transfer to the hutch would exceed the time permissible under anaesthesia, a cannula was implanted into the lateral ventricles and connected to a syringe pump installed in the hutch. Contrast agent would then be infused at 0.1 µl/min, matching the natural production rate of cerebrospinal fluid. Fly scans at different time points allowed assessing the contrast agent distribution (Fig. 1A, B). Connecting the cannulae while avoiding introducing air bubbles proved technically challenging, however, increasing the length of the sample mounting procedure, which had a detrimental effect on animal survivability. This revealed a need for real-time anaesthesia adjustment via remote-controlled injection or inhalation anaesthesia.

Artificial ventilation via tracheotomy was used, in addition, to induce a regular breathing frequency, which would allow for combined cardiac and respiratory gating. The Minerva breathing apparatus for rabbits available at the beamline was not suitable for use in mice, due to the larger dead volume. A dedicated mouse breathing apparatus (Hugo Sachs Minivent 845) was brought by the users, which was small enough to be mounted next to the sample on the rotation stage, allowing for minimal tube length and thus dead volume for artificial ventilation. Artificial ventilation successfully stabilized both cardiac and respiratory frequencies (Fig. 1C). As cardiac frequency would change along with breathing frequency, it was not possible to induce equal frequencies for both. Separate gating is therefore still necessary, barring further developments.

As infusion and tracheotomy were technically more challenging to perform and could only be performed by a limited number of members in the user team, the second night shift could not be used for *in vivo* experiments due to exhaustion. During this time, cadavers of mice previously scanned *in vivo* were scanned post-mortem after transcardial perfusion, to measure the changes in anatomy induced by death and fixation. Reconstruction of all data are still in progress.

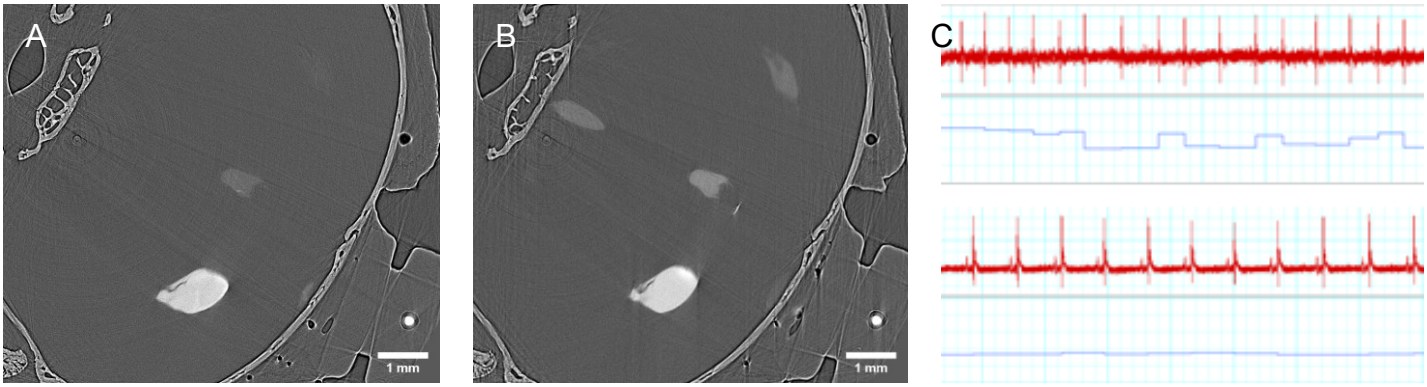

Fig. 1A, B: SRµCT slices showing infusion of contrast agent during the scans at different time points. Distribution from the injected lateral ventricle to the other CSF spaces can be observed. 1C: Electrocardiogram recording of freely breathing (top) and artificially ventilated mice (bottom). Red graphs indicate electrocardiogram recording, blue graphs indicate the heart rate.