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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:** Monitoring inflammation in stroke using combined high resolution magnetic resonance imaging and synchrotron radiation-phase computed tomography (SR-PCT) of the mouse brain	**Experiment number**: LS2292
Beamline: ID19	**Date of experiment**: from: April 2014 to: June 2014	**Date of report**: June 15, 2015
Shifts: 15	**Local contact(s)**: Vincent Fernandez, Lukas Helfen	*Received at ESRF:*

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Report:

Study#2- Preparation of fixed rodent brains for neuroinflammation imaging with Synchrotron-radiation x-ray phase microcomputed tomography

Complementary analysis are in progress for publication of this work.

Introduction

In previous works, we have developed an MRI method devoted to the imaging of neuroinflammation in stroke, based on magnetic labeling of macrophages with ultrasmall superparamagnetic particles of iron oxide (USPIO) (1-6). We then introduced Synchrotron-radiation x-ray phase computed tomography (SR-PCT) to map the distribution of USPIOs in the intact brain (7-9). Fixation is a critical step in preparing brain samples. Paraformaldehyde (PFA) is the most commonly used fixative for phase-contrast CT examination (10-13) ; however, it is toxic and carcinogenic (14). The aim of this study was to test the suitability of ethanol for preparing fixed rodent brain samples for SR-PCT imaging of USPIO-labeled macrophages.

Methods

Animals with neuroinflammation (stroke: N=3, heat injury: N=3, amyloid plaque: N=1, sham: N=1), that had received an i.v. injection of USPIOs for MRI experiments, were perfused with phosphate-buffered saline (PBS), followed by

infusion of PFA 4% (N=3) or ethanol 96% (N=5). The impact of ethanol concentration (range [24-96%]) was tested in 5 healthy mice. Fixed brains were then imaged in PBS using SR-PCT as described in (7). In brief, acquisitions were performed on beamline ID19 at ESRF at 19 keV selected from undulator radiation. An indirect detection-based detector (LuAg scintillator, visible light optics and 2048x2048 pixel CCD camera) was positioned 1-m from the sample to have phase contrast. Phase retrieval was performed using Paganin's method (15).

Results

With PFA fixation, regions with high nucleus density (e.g. plexus choroids) appeared hyperintense, while regions with low nucleus density (e.g. axonal fibers) appeared iso/hypointense (Figure 1B, 1E). Bright spots were observed in lesions with USPIO-labeled macrophages (Figure 1G). The same contrasts were observed with ethanol fixation (> 50%) (Figure 2B, 2E, 2G), except that white matter fiber tracts appeared hyperintense and could thus be tracked over thick virtual slices (Figure 2C, 2F). Quantitative measures such as contrast-to-noise ratio will be used to further compare both approaches.

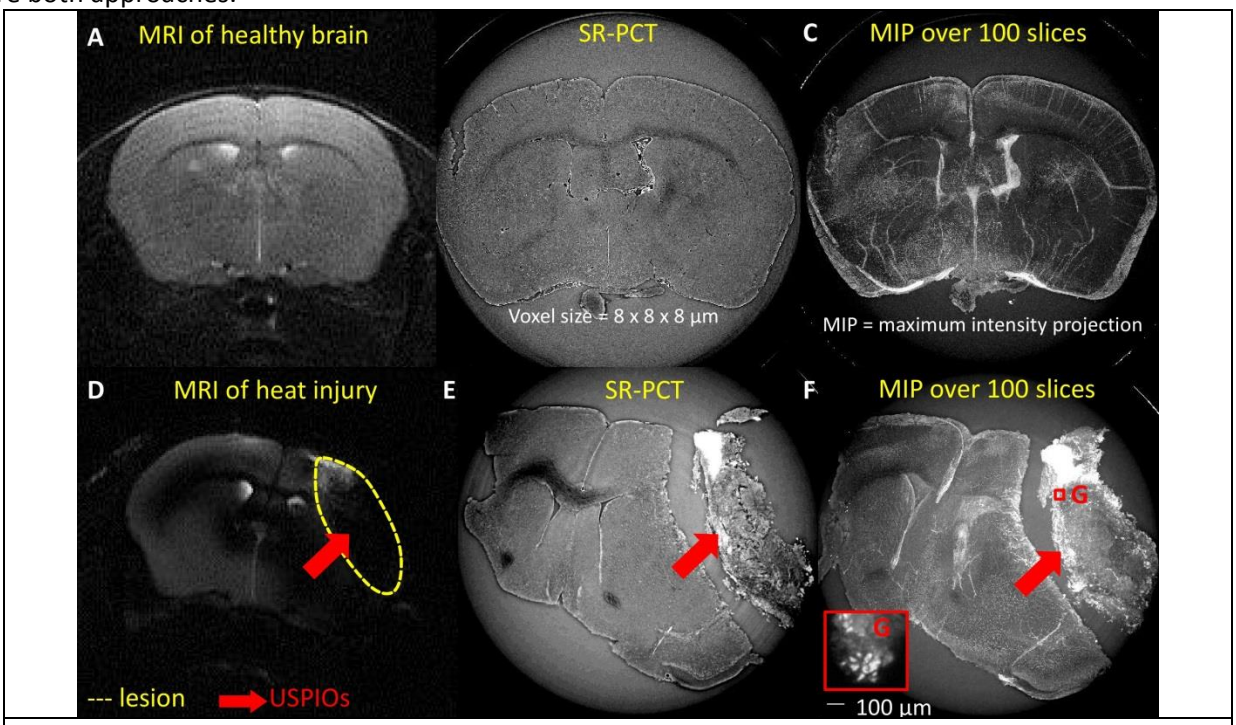

Figure 1- Fixation with PFA 4%

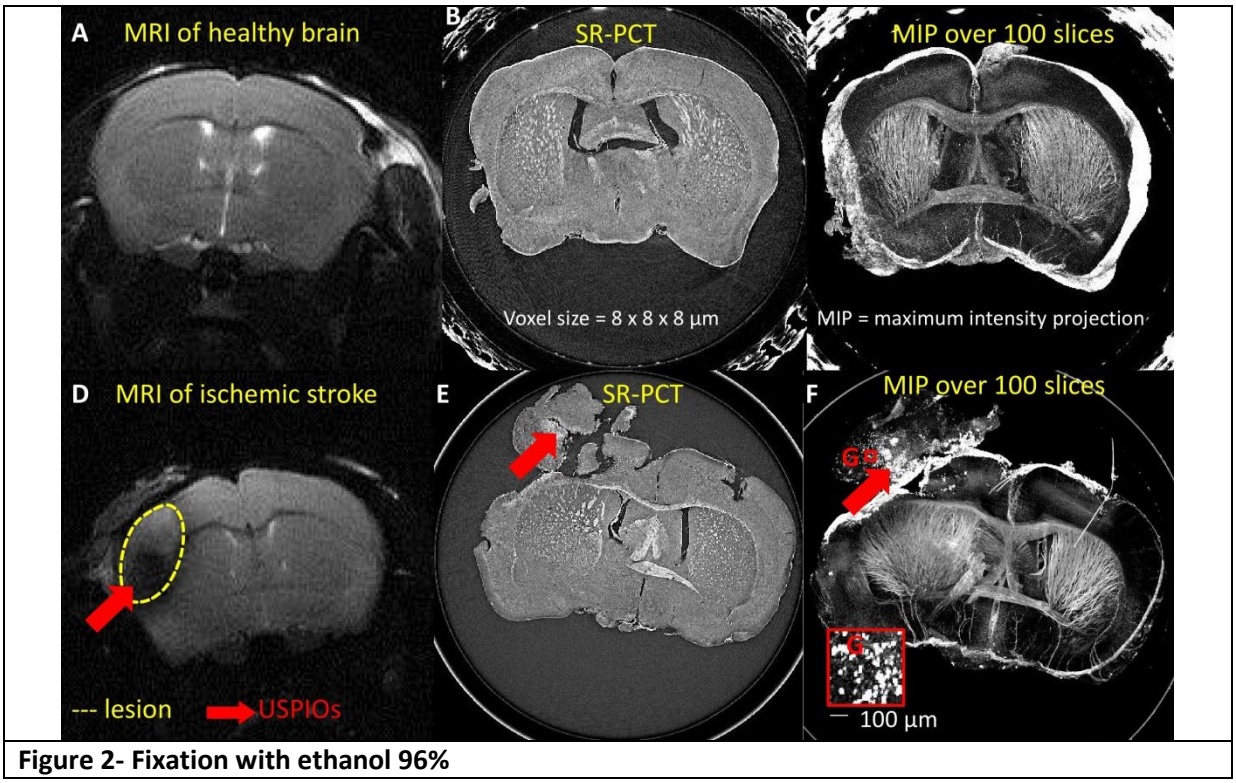

Figure 2- Fixation with ethanol 96%

Conclusion

High resolution imaging of fixed mouse brains is a powerful tool to detect pathophysiologic patterns without the need for sectioning or staining. Ethanol fixes proteins by dehydration and precipitation, and thus changes the density of soft tissues. Although it allows unprecedented fiber tracking in corpus callosum and striatum, this might become a limitation when looking for USPIO-labeled macrophages in these areas.

Acknowledgments

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