Imaging astrocyte reactivity using gluCEST

Julien Flament[1], Maria-Angeles Carrillo-de Sauvage[2,3], Julien Valette[2,3], and Carole Escartin[2,3]
[1]*U986 Inserm, MIRCen - CEA/Inserm, Fontenay-aux-Roses, France,* [2]*CEA-MIRCen, Fontenay-aux-Roses, France,* [3]*CNRS URA 2210, Fontenay-aux-Roses, France*

Target audience

This work should be of interest to people studying astroglial reactivity and neurodegenerative processes using proton spectroscopy and CEST imaging.

Purpose

Astrocytes are the most abundant glial cells and are key players in brain function. In response to pathological conditions such as neurodegenerative diseases or ischemia, astrocytes display morphological and physiological changes, which are referred to as astrocyte reactivity or activation [1]. Even if functional consequences of astrocyte activation remains poorly known (both protective and deleterious effects have been described), the presence of activated astrocytes could be a surrogate markers of alterations in the brain. Therefore, the possibility to monitor and characterize astrocytes status *in situ* is primordial and could offer a valuable tool for clinical research.

In order to evaluate astrocyte reactivity as potential biomarker, we used a model of selective astrocyte activation by overexpression of the cytokine ciliary neurotrophic factor (CNTF) [2]. It has been demonstrated that CNTF could trigger specific astrocyte activation without neuronal dysfunction [3]. Moreover, very reproducible variations of several brain metabolites were recorded using ^{1}H spectroscopy, especially increased myo-inositol (Ins) and total choline (Cho) and decreased N-Acetyl-Aspartate (NAA) and glutamate (Glu) [4].

In spite of the robustness of this result, it suffers from a lack of spatial resolution. Thus, we propose here to use Chemical Exchange Saturation Transfer (CEST) imaging of glutamate (gluCEST) [5] in order to precisely monitor spatial extension of astrocyte activation.

Methods

Animal model: Experiments were performed using a rat model (n=9) of astrocyte activation induced by stereotactic injection of a lentiviral vector encoding for CNTF (lenti-CNTF) into the right striatum [2]. The contralateral striatum was injected with a control lentiviral vector that encodes for beta-galactosidase (lenti-LacZ).

NMR experiments: MRS data were acquired on a horizontal 7T Agilent magnet in two voxels positioned in each striatum (Fig.1). A LASER (Localization by Adiabatic Selective Refocusing) sequence with echo time TR/TE=5000/25ms was used. Chemical shift localization error was less than ±5% over the 2-4ppm range. Glutamate concentration was calculated relative to total creatine (tCr) with good precision (Cramér-Rao lower bounds <5%) using LCModel.

GluCEST experiments: GluCEST images of 5 rat brains were acquired on the same magnet using Turbo Spin Echo (TSE) preceded by a frequency-selective continuous wave saturation pulse. Sequence parameters were: TR/TE=2000/50ms, 5 slices (1mm thickness) centered on stereotaxic coordinates used for lentivirus injections. The duration of saturation pulse was 1 s with an intensity B_1 of 3 µT applied at frequencies between -5 ppm to 5 ppm with a step of 0.2 ppm from bulk water. B_0 inhomogeneity was corrected using WASSR technique [6]. An unsaturated image was also acquired in order to calculate asymmetric Magnetization Transfer Ratio (MTRasym).

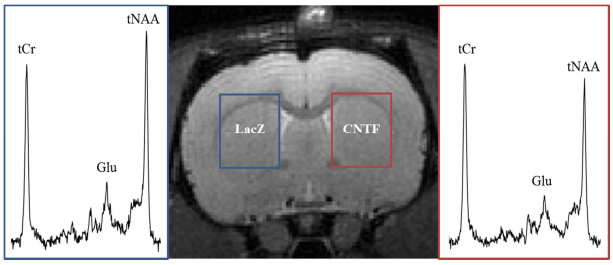

Fig.1: Position of voxels in the striatum injected with lenti-LacZ or lenti-CNTF (blue and red squares respectively) and corresponding spectra

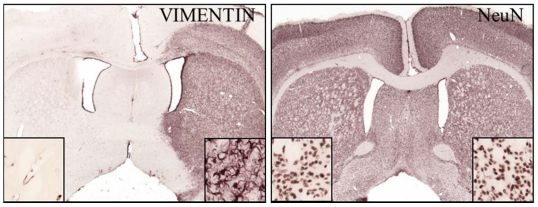

Fig.2: Vimentin and NeuN stainings of rat brain following injection of lenti-LacZ and lenti-CNTF (left and right striatum respectively). Overexpression of CNTF induced astrocyte activation (vimentin) without effect on neurons (NeuN)

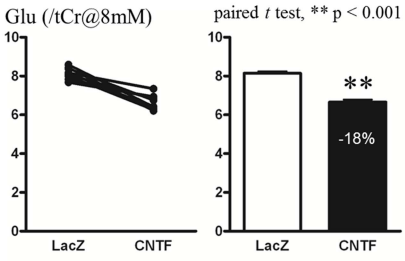

Fig.3: Injection of lenti-CNTF induced 18% decrease of Glu concentration measured by NMR spectroscopy (n=9)

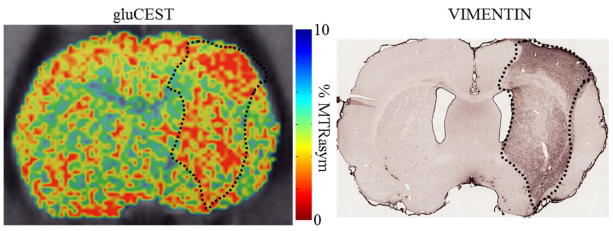

Fig.4: Example of gluCEST map and vimentin staining performed on the same rat. The area of activated astrocytes (dotted line) matched with the area of lower MTRasym

Results

Injection of lenti-CNTF induced selective and extended astrocyte reactivity in the right striatum, as demonstrated by the overexpression of the reactive marker vimentin (Fig.2). No toxic effect or abnormal feature was detected in neurons (Fig.2). Moreover, previous electrophysiological and histological studies demonstrated neurons integrity and even protective effect of lenti-CNTF injection following noxious insults [2, 3, 7].

Spectra acquired in LacZ and CNTF striata (Fig.1, blue and red squares respectively) showed a clear decrease of NAA and Glu in the lenti-CNTF side. A 18%-decrease of Glu concentration was measured in the whole animal cohort (Fig.3, n=9, paired t-test, **: p<0.001).

An example of gluCEST image is shown on Fig.4. The difference of MTRasym was visually evident between both hemispheres with a net decrease of gluCEST contrast in the CNTF-transfected striatum The average decrease of gluCEST contrast in a volume similar to spectroscopic voxels was 16% (n=5). Postmortem vimentin staining confirmed activated status of astrocytes in this area.

Discussion

Even if biological processes are still unknown, it seems that CNTF-induced astrocyte reactivity in absence of neuronal damage can be characterized by a significant decrease of Glu concentration. Importantly, the area of astrocyte activation (Fig.4, dotted line) matched precisely with the area exhibiting lower gluCEST contrast. This observation suggests that the decrease in glutamate concentration measured in the large MRS voxel is not due to a local CNTF effect of in the immediate vicinity of the injection site, but really occurs in the whole volume exhibiting astrocyte reactivity.

Conclusion

In this study, we demonstrated the potential of gluCEST imaging in order to accurately monitor spatial extension of astrocyte activation. Combined with metabolites quantification using spectroscopy, gluCEST imaging could be a valuable tool to follow *in vivo* progression of neurodegeneration or treatment efficiency.

References

[1] Escartin et al. *Mol Neurobiol* 2008; [2] Escartin et al. *J Neurosci* 2006; [3] Beurrier at al. *PLoS One* 2010; [4] Carrillo-de Sauvage et al. *Proc. ISMRM* 2013; [5] Cai et al. *Nat Med* 2012; [6] Kim et al. *Magnetic Resonance in Medicine* 2009; [7] Mittoux et al. *J Neuroscience* 2002.