Mn Distribution in rat hippocampus: Correlative use of Synchrotron X-Ray Microprobe and MEMRI

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INTRODUCTION It has been discovered that manganese (Mn) enhanced magnetic resonance imaging (MEMRI) can be used to trace neuronal connections activated following specific stimulation[1] or to enhance the brain region cytoarchitecture such as the hippocampus and the olfactory bulb.[2-3] The biological basis for the movement of Mn in tissues and its cellular distribution are still unclear. Furthermore, a major drawback to the use of Mn as an MRI contrast agent is its cellular toxicity. It raises the interest of identifying the brain cells targeted by Mn, in order to get better insights in MEMRI mechanisms and possibly to drive the development of new Mn-based contrast agents for pre-clinical applications.

MATERIELS AND METHODS ***Animals*****:** Twenty female 3-month-old Sprague-Dawley rats were used for this study. All procedures were performed under isoflurane anesthesia (2.5%). ***Mn injections*****:** Rats were divided in 2 arms and each arm in two groups (5 rats/group). **Arm 1:** intraperitoneal (IP) injection of $MnCl_2$ (30mg/kg, 100mM) (group 1); IP injection of NaCl (30mg/kg) (group 2). **Arm 2:** intracerebral (IC) injection of $MnCl_2$ (10µL, 50mM, 0,5µL/min) (group 3); IC injection of NaCl (10µl) (group 4) in the right hippocampus dentate gyrus. ***In vivo MRI:*** 24 or 48 h post-injection, T1-weighted images (Spin-echo, TR/TE=300/12ms) were acquired on a 7T MRI system (Bruker) using a surface/volume cross coil configuration (Fig 1A). ***Data analysis*****:** 5 regions of interest (ROI) were defined: background noise, cortex, dentate gyrus (DG), CA3, and CA1/2 in the Ammon's horn. The contrast to noise ratio (CNR) between cortex and DG, CA1/2, and CA3 was computed (Fig 2A). The MEMRI contrast for an ROI was obtained by comparing the CNR obtained in animals with and without Mn. After MRI, animals were sacrificed. Brains were quickly removed, frozen in isopentane and stored at -80°C. Thin brain cryosections (10 or 20 µm) of the ventral hippocampus structure were obtained. ***Synchrotron imaging*****:** sections were mounted on laser micro-dissection slides. X-ray fluorescence (SR-XRF) microanalysis was performed on one area per brain using European Synchrotron Radiation Facility (ESRF) microprobe end-station to map the spatial distribution of calcium (Ca), Mn, iron (Fe) and zinc (Zn). The size and the energy of the microprobe spot were 5x15 μm^2 and 14.4keV, respectively. Acquisition time was 7s/pixel. ***Histology*****:** Immunofluorescence was performed on sections adjacent to those analysed by SR-XRF. Neurons and astrocytes were labelled using antibodies against NeuN and GFAP, respectively.

RESULTS Fig. 1A shows typical MRI images. Inter-animal variabilities of CNR in the hippocampus after systemic and intracerebral injection of Mn are 7% and 11%, respectively. In Arm 1, significant MEMRI contrasts are observed in the DG (+24%), CA3 (+19%) and CA1/2 (+11%) (Fig. 2A). In Arm 2, the MEMRI contrast raises to +134% in DG, +96% in CA3 and +90% in CA1/2. SR-XRF representative distributions of Mn, Ca and Zn within the hippocampus are shown in Fig. 1B. The average Mn concentration within the DG/CA3 is 192 ppm (µg/g) in control whereas it is 2.8 ppm in the surrounding tissues. In Arm 1, Mn in DG/CA3 rises to 345 ppm. This figure becomes 3880ppm in the contralateral hemisphere of Arm 2. The latter also displays very high Mn concentration (16100 ppm) in the hippocampal fissure (HF). Zn distribution follows the hippocampal mossy fibers, which project from dentate granule cell to dentate hilus and CA3. The Mn location parallels that of Zn, particularly in Arm 2. Interestingly, the Zn signal in CA3 and DG is clearly higher in control animals than in those that received Mn, while Zn distribution remains similar. A concomitant decrease of Fe and Zn in the DG/CA3 upon IP injection of Mn is observed (not shown). This decrease is more pronounced in IC injected animals. Surprisingly, while MEMRI signal is enhanced in same hippocampal regions for Arms 1 and 2, SR-XRF reveals, for the latter, a preferential location of Mn in the HF concomitantly to Ca (Figs 1B and 2D). The immunofluorescence analysis using NeuN (green) or GFAP (red) indicates that HF is composed of astrocytes (Fig. 2B, 2C).

DISCUSSION This study provides the first Mn maps at a cellular scale in rat brain hippocampus after systemic and IC administration of $MnCl_2$. The hippocampal distributions of Mn in SR-XRF images and in the MEMRI images are in excellent agreement. In Arm 2, SR-XRF imaging reveals that Mn is preferentially located in the astrocytes found along the HF. It corresponds to a hypo-intense region in MEMRI images. This hypointense signal could be ascribed to a high concentration of Mn and/or a compartmentalization of Mn in cells. The observed Zn location (within DG and mossy fibers along CA3) corresponds to what has been reported in the literature[4-5]. SR-XRF images suggest that Mn compete with Zn and Fe which are found reduced in IP and IC animals. We can suppose that Mn location follows that of zinc in synaptic vesicles detected in the mossy fibers projections from granule cells to hilar neurons and pyramidal CA3 cells[5]. The high local Mn concentrations obtained after IC injection might leads to saturation in the hippocampus with excess detoxified by astrocytes predominantly found in the HF.

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