Effect of manganese on rat hippocampus metabolism: a 1H HRMAS study

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INTRODUCTION Manganese (Mn) Enhanced Magnetic Resonance Imaging (MEMRI) can be used for different applications such as tracing neuronal connections or functional imaging[2-3]. However, mechanisms of Mn transport and its toxicity in tissues are still unclear. The aim of this study is to identify Mn induction of metabolism perturbations to get better insights on the impact of MEMRI on brain function using High Resolution Magic Angle Spinning (HRMAS) NMR spectroscopy.

MATERIELS AND METHODS ***Animals*****:** sixteen 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 groups (8 rats/group). An intracerebral (IC) injection was performed in the dentate gyrus of the right hippocampus (Fig. 1A): group 1 received $MnCl_2$ (10µL, 50mM, 0.5µL/min) and group 2 was control (10µl PBS – vehicule). ***In vivo MRI:*** 24h 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. 1B, C). ***Sample preparation*****:** After MRI, animals were quickly euthanized by decapitation. Brain was removed and the whole hippocampus was dissected on ice at 4°C. Ipsi- and contralateral hippocampus were differentiated and further divided in 3 equal parts: dorsal, intermediate and ventral hippocampus (DH, IH and VH, respectively) and finally rapidly frozen in liquid nitrogen. ***HRMAS*****:** ^{1}H HRMAS NMR spectra were acquired with an Avance 400 Bruker at 400MHz and a 4mm 1H-13C-31P probehead. Samples were spun at 4KHz and temperature was maintained at 4°C during the acquisition. A CPMG sequence with a 30ms echo time was used to attenuate the broad signal of lipids and macromolecules. NMR data were quantified using jMRUI software (www.mrui.uab.es/mrui/). Results are expressed as mean±Standard Deviation. Unpaired Student t-tests were performed to compare absolute values of metabolite concentrations with and without Mn. Significance was: * p<0.05, **p<0.01; *** p<0.001.

Fig. 1

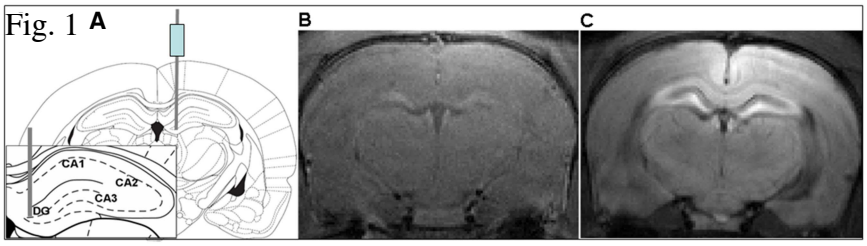
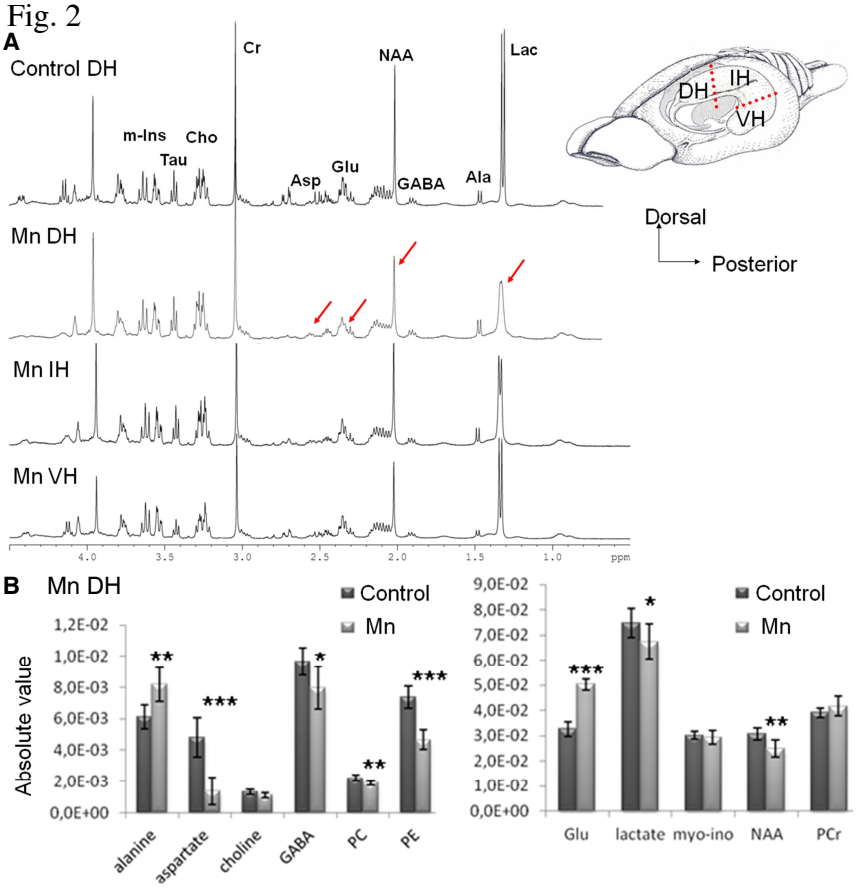


Fig. 2



RESULTS Animals that received Mn exhibit a higher MR signal in both sides of the hippocampus (Fig. 1C) than animals injected with vehicle (Fig. 1B). Two effects were observed in HRMAS NMR spectra. (**i**) In the ipsilateral DH, some resonances were broadened due to the Mn paramagnetic effect (Fig. 2A), mainly lactate, NAA, glutamate and aspartate resonances, while peaks like taurine were not affected; (**ii**) in the injection site (ipsilateral DH), an important impact on cerebral metabolism was observed (Fig. 2B). Both effects were not identical in each of the three parts of the hippocampus, and not identical in ipsi- and contralateral hippocampus (data not shown).

DISCUSSION Organic acids are known to chelate Mn. This could explain the broadening effect of Mn on resonances in HRMAS NMR spectra[4]. Moreover both metabolism and broadening variations along hippocampus suggest that these effects are proportional to the Mn concentration. We observed a disturbance of glutamine-glutamate-GABA cycle, consistent with literature[5]. An astroglyosis is revealed by the disturbance of organic osmolites (choline, myo-inositol…)[6]. To conclude, these preliminary data are encouraging and could shed some light on how Mn interacts with brain cells metabolism.

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