MEMRI of organotypic rat hippocampal slice cultures

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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[1-3]. However, mechanisms of Mn transport and Mn synaptic transmission are still unclear. The aim of this work was to develop a hippocampal organotypic slice culture in an open chamber to allowed slice manipulation (e.g. injection, microscopy and electrophysiology) and high resolution MRI. This slice culture should enable the study of Mn transport mechanisms and synaptic transmission.

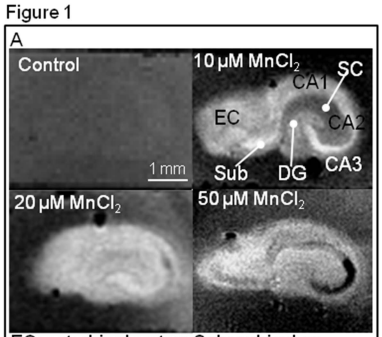
METHODS ***Animals***: Post natal day 6 Sprague Dawley rats were used for this study. ***Slice preparation and culture***: Hippocampi were dissected and coronal sections (300 µm) were sliced in an artificial CSF (aCSF) at 4ºC [4]. Slices were cultured at the interface medium/air on a membrane insert previously coated with 0.1 mg/mL poly-L-lysine (incubation at 37 ºC, 5%CO_2) for 4 days (D4). ***Mn injection***: Experiment 1: On D5, Mn was added at 10, 20 and 50 µM to the culture medium for 24h. On D6, The membrane insert was cut around the slice, placed in a perfusion chamber and fixed with a plastic anchor. Experiment 2: The slice (D5) was placed in a perfusion chamber (see experiment 1) and in a microscope (perfusate = aCSF at 37 ºC). A solution of 1mM Mn was microinjected in the CA3 (injection pressure = 260 hPa; injection time = 0.3 s) and the injection was confirmed with 1 mg/mL Lucifer yellow in the solution (Fig 2A). ***MEMRI***: 24h (experiment 1) or 25, 40, 55, 70, 85 and 100 min (experiment 2) after Mn injection, T_1 weighted images (FLASH, TR=25ms, TE=4.65 ms, voxel size: 50x50x50 µm) were acquired on a 11.7T MRI system (Bruker) using a surface/volume cross coil configuration. For experiment 1, T_1 map (saturation recovery, 5TR=[300-12500 ms], TE=10 ms, voxel size: 0.2x0.2 mm) were acquired as well, 24h after Mn addition to the medium. Slices were continuously perfused with aCSF at room temperature. ***Data analysis***: For the T_1 map (experiment 1), regions of interest (ROI) surrounding the slice were manually drawn. T_1 values were converted to changes in Mn concentration[5] from control using a r1 of 6.5 $mM^{-1}.s^{-1}$ [6] (Δ Mn concentration). Results are expressed as mean ± Standard Deviation. For experiment 2, ROIs were manually drawn for each time point of the MRI follow-up using a rat brain atlas as a visual reference: *Cornu Ammonis* (CA1/2 and CA3), dentate gyrus (DG), Schaffer collaterals (SC) and the background noise (Fig 2C). The signal to noise ratio (SNR) was computed for each ROI (Fig 2C).

RESULTS MRI images revealed excellent contrast in presence of 10 µM Mn in the medium. Areas of the hippocampus can be readily detected compared to control (Fig. 1A). At 20 µM there is less grey/white matter contrast and at 50 µM, there is signal loss. The T_1 decreases in presence of 10, 20 and 50 µM of Mn (-41, -64 and -82%, respectively; Fig 1B). The calculated Mn concentration in the slice increase proportionally with Mn concentration in the medium (y=6.5x-6.2; R^2=0.9978, Fig 1B, graph not shown). Fig 2B shows the MEMRI before and after Mn injection into the CA3 region. The SNR evolution over time after Mn injection revealed a SNR increase in ROIs from CA1/2, DG and SC. (Fig 2C). There is a 8% SNR increase in the CA1/2 40 min after Mn injection and a 15% increase in the SC, 100 min after the injection which is interpreted to be due to Mn transport from the injection site.

DISCUSSION An organotypic rat hippocampal slice culture has been developed to understand mechanisms associated with MEMRI. 10 µM Mn in the culture medium gave excellent contrast compared to 20 and 50 µM. At 50 uM a lower signal was likely due to a reduction in T_2 due to the high Mn concentration[7]. The calculated Mn concentration in the tissue (50 µM) in presence of 10 µM in the medium corresponds to the concentration found in the hippocampus after a systemic injection of 30 mg/Kg, 100 mM in rat[7]. The tract tracing experiment after injection into CA3 revealed Mn accumulation in the CA1/2 and then in the SC, suggesting a first Mn transport due to short range local connections and then axonal transport to more distant sites. The hippocampal slice will enable the use of a variety of manipulations to understand the mechanism of Mn transport in neurons and across synapses.

REFERENCES [1]Lin and Korestky, MRM, 38:378-388, 1997; [2]Aoki et al., Neuroimage, 22:1046-1059, 2004; [3]Daoust et al., Neuroimage, 96:133-42, 2014; [4]Fuller and Dailey, CSH protocol, 2007; [5]Silva et al., NMR biomed, 17:532-543, 2004; [6]Chuang et al., Magn Reson Med, 61(6):1528-32, 2009; [7]Daoust et al., Neuroimage, 64:10-18, 2013.

Figure 1



EC: entorhinal cortex; Sub: subiculum

B

Mn concentration in the medium (uM)	T_1 (s)	Δ Mn concentration in the tissue (uM)
0	2.11 ± 0.1	-
10	1.25 ± 0.06	49.7
20	0.77 ± 0.06	126.3
50	0.39 ± 0.03	320.6

Figure 2