

MRS of neuronal progenitor Cells in vivo

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Introduction:

The discovery of ongoing neurogenesis—the birth of new neurons—in the adult brain has been one of the most remarkable and surprising findings during the last decades. Lately Manganas et. al used ¹H-MRS to investigate neural progenitor cells (NPCs) in vitro and detected a peak at 1.28 ppm which they did not find in other cultured neural cell types. This study aims to inquire this resonance by measuring the ¹H MRS signal of living NPC cell cultures.

Methods:

Mice with C57BL/6 background (Charles River, Milano, Italia) 2 ± 0.5 months of age were used. Hippocampi were removed from brains chilled in PBS and digested by Earle's balanced salt solution (EBSS), containing DNase, 0.2 mg/ml cystein and EDTA. Tissue pieces were washed and dissociated. Cell pellets were finally placed in growth medium. Cells were serially subcultured by mechanical dissociation every 4-7 days. MRS measurements were performed in a 9.4T animal scanner (Bruker, Rheinstetten, Germany). Directly before scanning the cultured NPCs were concentrated by centrifugation to obtain 18-20 million cells in 2ml growth medium. The cells were placed in a two compartment phantom surrounded by a buffered solution containing 200mM NA-formate and 2mM DSS for referencing and phasing. During the measurement the temperature of the phantom was kept around 38°. Single voxel spectra were obtained from a 64µl Volume containing the cultured NPCs as well as a portion of the reference solution. Spectra with 256 averages at TE=10ms and TR=5s were acquired from the growth medium without NPCs, the growth medium containing NPCs and the medium after extracting the cells respectively. Additional spectra were acquired at TE=144ms. The shim in all measurements was 6Hz or better.

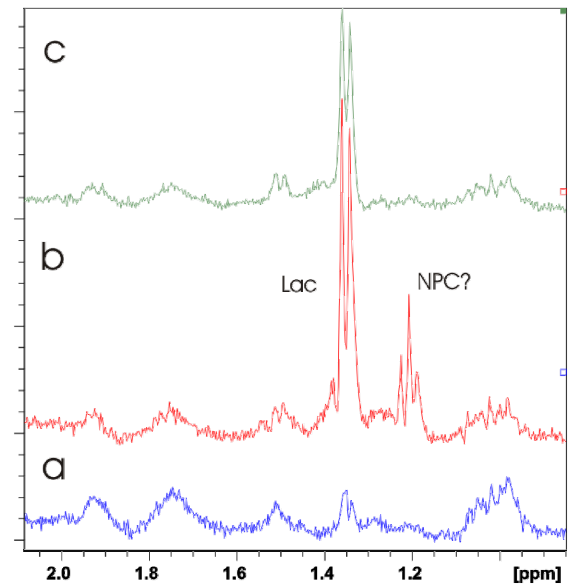


Fig 1: PRESS spectra (TE=10ms) of growth medium (a), growth medium with NPC (b) and growth Medium after NPC (c)

Results:

Compared to the spectra of the growth medium without NPCs, the spectrum containing NPCs shows two additional prominent signals at around 1.33 and 1.21 ppm respectively. The Peak at 1.33 ppm is Lactate and has a 180° phase shift at TE=144ms whereas the triplet's phase seemed unchanged. This triplet show similar spectral patterns to ethanol. While the Lactate signal was also measured in the growth medium after the NPCs had been removed, the triplet at 1.21ppm was not detected here. No resonance signal was measured at 1.28 ppm.

Discussion:

We were able to measure living NPCs in growth medium and detected a prominent triplet at 1.21 ppm that could be linked to the NPCs. However, we could not detect the resonance at 1.28 ppm and the origin of the 1.21 ppm line is unclear. The position and behaviour of the resonance at TE=10ms and TE=144ms suggest, that this line might correspond to ethanol, which has already been detected in cell cultures by others. But how this substantial concentration (> 1 mM) of ethanol entered the cell culture needs to be further investigated.

References:

- [1] Manganas LN et al, Science (2007) 318:980-985
- [2] Römer et al BioEssays (2008) 30:806-810
- [3] Gritti A, J Neurosci. 1996 Feb 1;16(3):1091-100

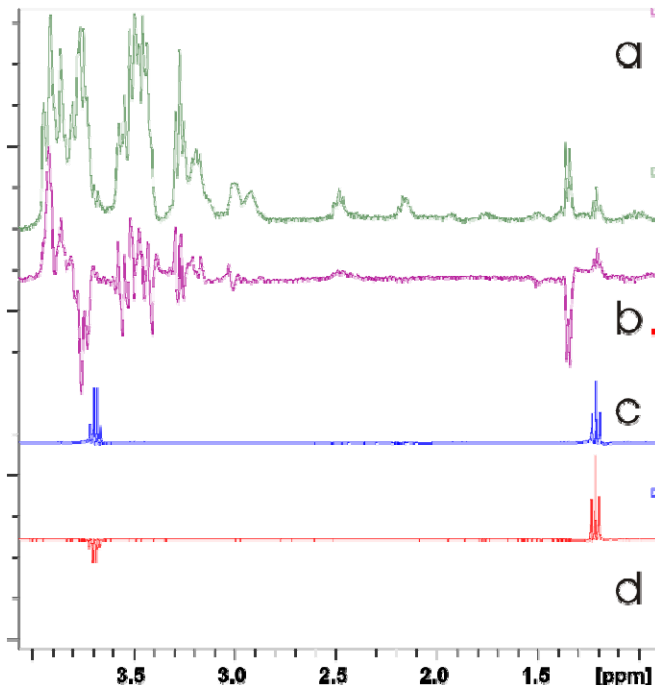


Fig 2: PRESS spectra of NPC (in growth media) at TE=10ms (a), NPC at 144 ms (b), Ethanol at TE=10 ms and 144 ms (c and d)