In Vivo High Resolution Localized Two Dimensional Magnetic Resonance Spectroscopy in Mouse Brain

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Introduction

Localized two-dimensional magnetic resonance spectroscopy (2D MRS) is revolutionizing the *in vivo* studies of brain metabolites due to improved spectral resolution and unambiguous assignment opportunities. Despite the large number of transgenic mouse models available for neurological disorders, localized 2D MRS has not yet been implemented in the mouse brain due to size constraints[1]. In this study we optimized a localized 2D proton chemical shift correlated spectroscopic sequence at field strength of 9.4T to obtain highly resolved 2D spectra from localized regions in mouse brains *in vivo*.

Methods

All measurements were performed using a 9.4-T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. Pilot images for voxel positioning were acquired using the RARE sequence. The MRS voxel was located in the hippocampus/cortex region (4x4x1.7 mm^3; 27 µl – Fig. 1a). Field homogeneity was optimized using the Fastmap sequence, which typically yielded a water linewidth of ~16-20 Hz in live mouse brain.

For 2D MRS, the 1D PRESS protocol was modified based on the work by Thomas et al. (2001),[2] to yield a localized 2D shift correlated spectroscopic sequence (L-COSY) for the 9.4T MR spectrometer. The sequence consists of three RF pulses (90°, 180°, 90°), slice-selective along 3 orthogonal axes. The last slice-selective 90° RF pulse also served as a coherence transfer pulse for the L-COSY spectrum necessary for correlating the metabolite peaks in the second dimension. 2D spectra were recorded using TR=1500 ms, TE=15 ms, 2048 complex points along F2, and 192 points along F1, with a spectral width of 11 ppm, and 20 averages per excitation step. Total scan time for a typical 2D measurement was ~96 minutes.

Results and Discussion

In vivo localized 2D MRS was performed in the mouse brain after positioning a voxel in the hippocampus/cortex region - a region which is commonly affected in neurodegenerative disorders such as Alzheimer's disease. Figure 1b shows a typical example of a spectrum obtained from a 27 µl voxel positioned in the hippocampus/cortex. From the 35 or so metabolites present in the brain[2], 16 could be detected, 13 of which in a single measurement. Of interest is the fact that N-acetyl-aspartyl-glutamate (NAAG), glutathione (GSH), Lactate (Lac), γ-aminobutyric acid (GABA), threonine (Thr) and a number of other metabolites that are commonly lost in 1D spectra due to spectral overlap, can be clearly distinguished in our 2D spectra (Fig. 1b).

Conclusion

The combination of the optimized 2D sequence, high field strength, strong gradient system, efficient water suppression and the use of short echo time allowed clear detection of cross-peaks of up to 16 brain metabolites allowing their unambiguous chemical shift assignments *in vivo*. To our knowledge this is the first application of 2D MRS in mouse brain, at high field. The L-COSY method yields consistent, reproducible results, and thus allows the clear and unambiguous identification of multiple brain metabolites from a single measurement. Such information will prove invaluable in future studies of brain disorders in mouse models.

References: [1]Méric and Autret et al. Magn Reson Mat Phys Biol Med 2004;17:317-338; [2]Thomas and Yue et al. Magn Reson Med 2001;46:58-67. [3]Govindaraju and Young et al. NMR Biomed 2000;13:129-153; [4]Behar and Ogino. Magn Reson Med 1993;30:38-44.

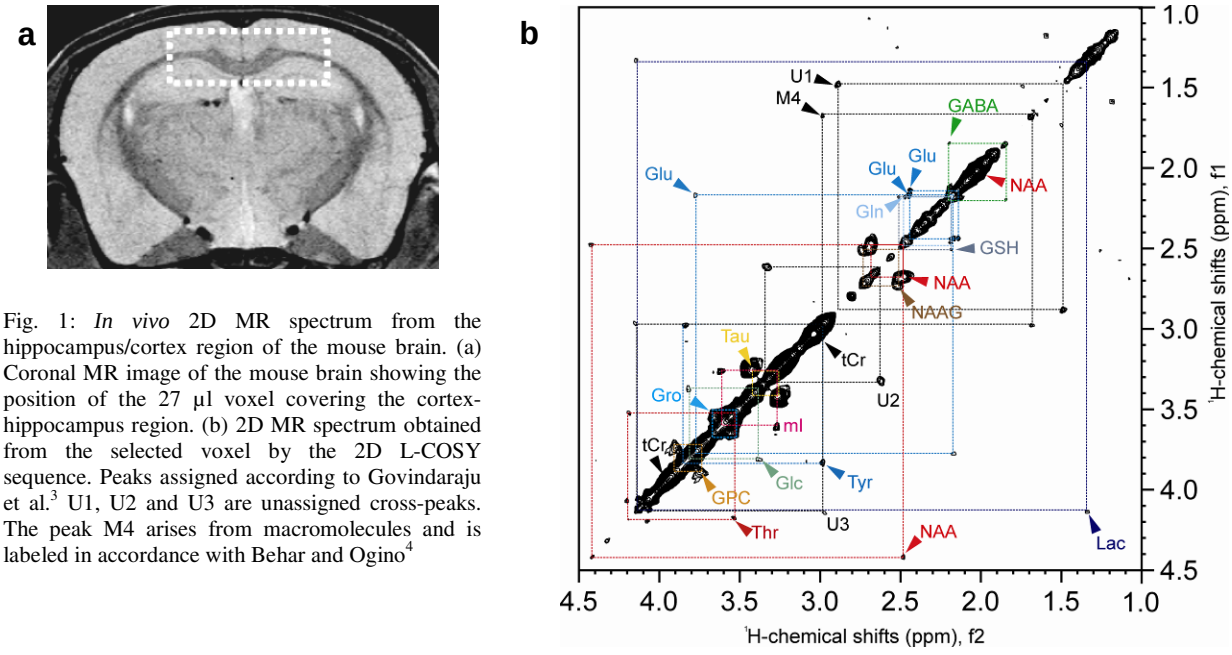

Fig. 1: *In vivo* 2D MR spectrum from the hippocampus/cortex region of the mouse brain. (a) Coronal MR image of the mouse brain showing the position of the 27 µl voxel covering the cortex-hippocampus region. (b) 2D MR spectrum obtained from the selected voxel by the 2D L-COSY sequence. Peaks assigned according to Govindaraju et al.[3] U1, U2 and U3 are unassigned cross-peaks. The peak M4 arises from macromolecules and is labeled in accordance with Behar and Ogino[4]