# Regional alterations of the brain macromolecule resonances investigated in the mouse brain using an improved method for the pre-processing of the macromolecular signal

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**Introduction** An accurate quantification of the macromolecule (MM) contribution in ^{1}H MRS spectra acquired with short echo time (TE) is mandatory for reliable metabolite quantification especially at high magnetic field strengths due to the increased spectral resolution of MM [1]. However, MM signals are not acquired in each animal as it is time consuming and needs several pre-processing steps. Currently, an inversion-recovery (IR) sequence is the gold standard for the MM acquisition but due to different $T_1$ relaxation times of brain metabolites, a residual contribution still needs to be removed from the MM signal. Up to now HLSVD [2] is the only proposed pre-processing method. Consequently the same MM signal is commonly used to quantify spectra in different regions of the brain and even in different species. Moreover, although the metabolite profile regionally varies in the mouse brain [3], regional alterations of the MM signal have never been investigated.

Therefore the aim of this study was to develop a more flexible and improved method than HLSVD to remove residual metabolite signals from the MM signal and to investigate regional alterations of the MM resonances in the mouse brain.

**Methods** MRS experiments were performed on a horizontal 14.1 T magnet (Varian/Magnex) with a custom-built quadrature transceive RF coil. After FASTMAP shimming, localized ^{1}H spectra were acquired in three mice in two different brain regions (the striatum STR, VOI=8µl and the hippocampus HIP, VOI=6.75µl) using SPECIAL [4] with 512 averages, TE=2.8ms, TR=4s. For the MM signal acquisition, metabolite-nulled spectra were acquired in the same volumes and in a region with mixed tissues (VOI=27µl) by using an adiabatic inversion RF pulse before the SPECIAL sequence (TE=2.8ms, TR=2.5s, TI=775ms). Identification of residual metabolite signals in the MM spectrum was performed using a series of IR acquisitions (TI: 600 to 900ms) at shorter and longer TE (TE=2.8 and 40ms) [1, 5]. Residual metabolite signals were then removed using AMARES [6], where constraints on the peak frequency, phase, linewidth and amplitude were manually set to fit the residual metabolite signals that were to be removed. The setting of the constraints was based on prior knowledge of the frequency and phase of the residuals. Iterative fitting was then performed to improve accuracy of the metabolite residuals fit at each step after redefining stronger constraints on amplitude and linewidth. For comparison, the metabolite residuals were also removed with HLSVD.

Metabolite concentrations were calculated with LCModel [7] using a simulated basis set of 21 metabolites and the corresponding measured MM signals or a common rat brain macromolecule signal. Water signal was used as internal reference for absolute quantification.

**Results and discussion** Overall, high quality metabolite and metabolite-nulled spectra were consistently acquired (Fig. 1). Residual metabolite signals from the metabolite-nulled spectra were efficiently removed with AMARES after a careful setting of parameter constraints that allowed optimal fitting of the residuals. In comparison, HLSVD did not allow to optimally remove the metabolite residuals as no constraints or prior knowledge could be set, which resulted in visually detectable residual over- or under-estimations and baseline distortion (Fig. 2) that is likely to affect the subsequent metabolite quantification accuracy of the creatine, taurine, glutamate and the overlapping metabolites at those frequencies. After removing the metabolite residuals with AMARES, MM spectra acquired in the different brain regions visually expressed no major differences, which is in agreement with what has been found in the rat brain [8]. Metabolite quantification differences when the specific regional MM or the mixed region MM signal was used were below 10% for most metabolites (Fig. 3). The higher but not statistically significant differences found for Asp (15%), Gly (15%), GPC (20%), NAAG (30%), Asc (40%), PCho (40%), PE (40%) could be explained by their lower contributions in the spectra, higher correlations and Cramér-Rao lower bounds and the slight differences in the Glx residual signal removal of the MM signals at 3.75 ppm. A similar quantification consistency could be observed for the results obtained with the rat MM signal.

**Conclusion** We developed a novel and more accurate method than HLSVD to remove residual metabolite signals from the MM signal. Moreover, as no significant effects have been detected on metabolite quantification due to potential MM signal regional alterations in the mouse brain and to alterations between mice and rats MM signals, we conclude that a macromolecule signal acquired in a healthy mouse can be used for a general quantification of mouse and rat brain spectra.
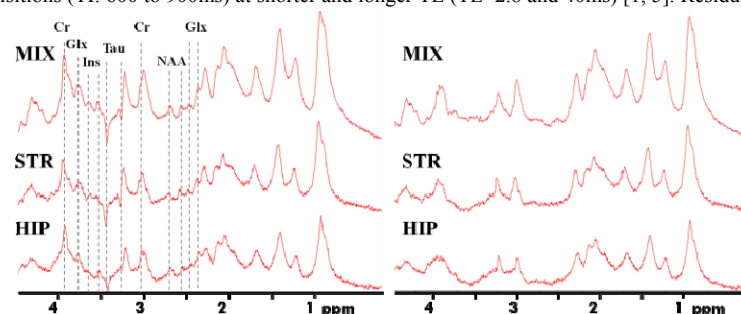


**Figure 1** *Metabolite-nulled spectra before (left) and after (right) removing the residual metabolite signals with AMARES. Dotted lines in the left image indicate residual metabolite resonances; these can no longer be observed in the figure on the right.*
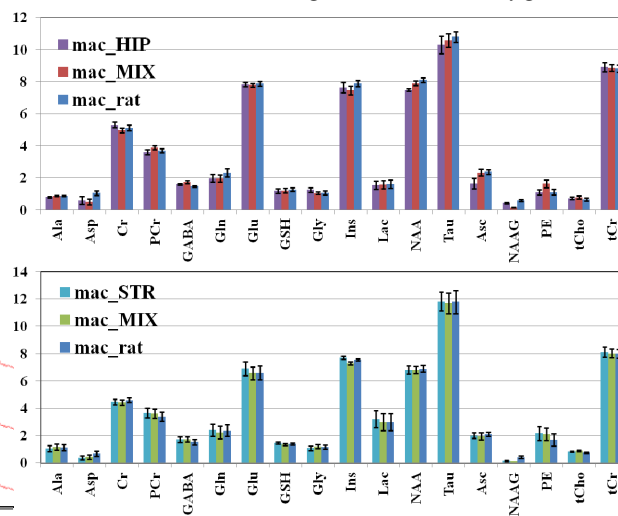


**Figure 2** *Metabolite-nulled spectra processed with HLSVD and AMARES for removing metabolite residual signals. Dotted lines indicate sub-optimal metabolite signal suppression with HLSVD.*



**Figure 3** *Metabolite quantification results of the hippocampus (upper panel) and striatum (lower panel) obtained using the specific regional, the mixed region and the rat macromolecular signal. No significant difference in quantification was observed for any metabolite.*
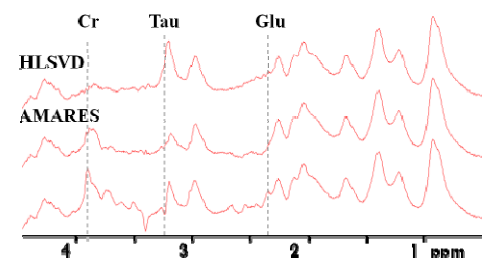
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