In vivo 1H MRS quantification of Alzheimer disease in frontal hippocampus of mice with and without inversion recovery to assess the macromolecular contribution

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Introduction

Magnetic Resonance Spectroscopy (MRS) provides non-invasively metabolite information for the diagnosis of cancer and other metabolic diseases. This information is estimated using quantification methods that give the concentration of the metabolites present in the tissue under investigation. Several methods have been developed to estimate metabolite concentrations [1,2,3], by using time or frequency domain techniques to fit the model signals (metabolite profiles) to the spectra under analysis. Alzheimer disease has been studied using ^{1}H MRS in different brain regions, where metabolite changes have been observed [4,5]. In this study, we analyze the metabolite estimates in the hippocampus of mice for both control and APP.V717I transgenic [6] mice animals taking into consideration the macromolecular contributions, we compare the situation when the background signals have been measured individually and when they are computed inside the quantification method using splines. On the other hand, the hippocampus has a small size and is located near to air cavities and the skull, which affects spectral quality making metabolite estimation to become a challenge.

Methods

Animals: Twelve control and APP.V717I transgenic wild-type mice of age 16-18 months were used for this study. Mice were anesthetized by using 1.25% isoflurane and their heads were immobilized during experiments. Body temperature was measured using a rectal thermometer, the air temperature was maintained around 31°C. Breathing was also measured during the experiments to control diaphragm movements that could influence the measurements. **[1]H MR Spectroscopy:** Single Voxel ^{1}H MR Spectroscopy (SVS) of *in vivo* mice brain from the frontal hippocampus was performed on a Bruker Biospec 9.4 T small animal MR scanner using a 60mm linear body resonator as transmitter combined with a circular polarized ^{1}H mouse brain surface coil for signal reception (Bruker BioSpin, Ettlingen, Germany). *In vivo* SVS spectra were obtained using the PRESS pulse sequence implemented in a predelay Outer Volume Suppression (OVS) as well as VAPOR for water suppression. Short echo-time MRS parameters were: TR=4s, TE=12ms, SW= 4 KHz and 256 averages. The *in vivo* spectrum from macromolecules was measured using a 1ms Hermitian inversion pulse. The inversion time and repetition time were 800ms and 3s, respectively, with 1024 averages. The inversion time was optimized experimentally. Spectra were corrected for B0 eddy currents as well as B0 drift using the Bruker built-in routines. Shimming was performed using FASTMAP. SVS and FASTMAP VOIs were 3x1.75x1.75 mm^3. The linewidths of the unsuppressed water were between 20-25 Hz.
Data processing: In vivo ^{1}H MRS signals were analyzed using AQSES [1] and jMRUI [2]. AQSES is able to estimate the background modeling using splines. Preprocessing of data consisted in time circular shift, phase correction, water removal and normalization, which were performed in jMRUI. The AQSES time domain quantification method was applied to estimate metabolite concentrations by fitting a linear combination of metabolite profiles to the experimental data. Two metabolite basis sets were used for analyzing the data: a) A set of 14 *in vitro* metabolites, b) A set of 14 simulated metabolites, obtained from jMRUI. The following metabolites were included in the basis sets: alanine (Ala), aspartate (Asp), creatine (Cre), γ-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycerolphosphorylcholine (GPC), phosphorylcholine (PCh), *myo*-inositol (Myo), lactate (Lac), N-acetylaspartate (NAA), phosphocreatine (PCr) and taurine (Tau). Additionally, the spectra of macromolecules (MM) for each individual mouse was measured experimentally using an inversion recovery pulse as described above, which was then included in the basis set for quantification. Statistical analysis of spectra and metabolite estimation was done in Matlab®.

Results

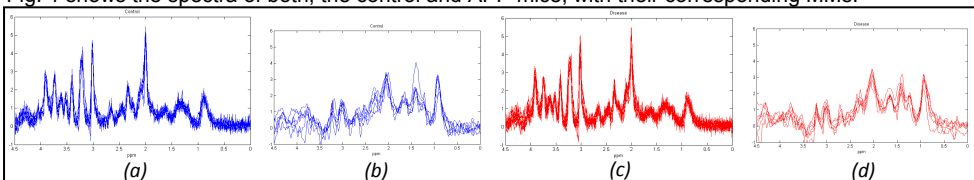Fig. 1 shows the spectra of both, the control and APP mice, with their corresponding MMs.

Fig. 1 SVS in vivo ^{1}H MR spectra of control (a) and disease (c) mice obtained from the frontal hippocampus. (b) and (d) show the corresponding macromolecular signals measured by inversion recovery.

In Fig. 2 we evaluated the results of quantification of the mean spectra, in particular the influence of the background estimation and spectral groups. In Fig 3 we explore the possible differences in the metabolite estimates in the two classes.
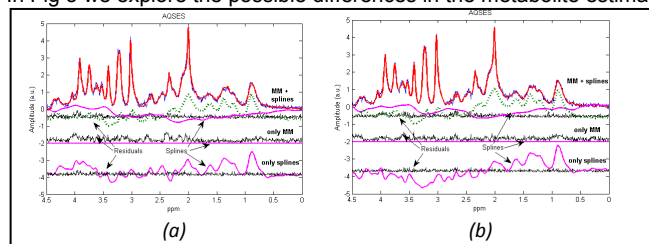

Fig. 2 Fitting was performed in AQSES using the mean of the MM signals for each group together with splines to account for the background contamination. Results for control (a) and disease (b) mice with MM and splines, only with MM and only with splines
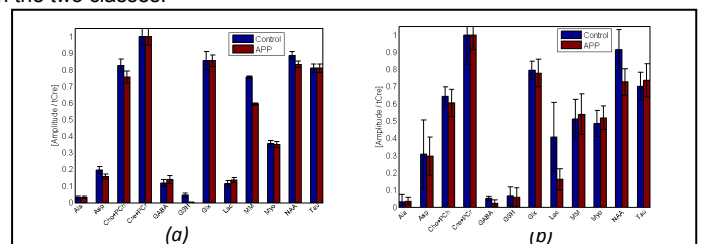
Fig. 3 (a) Metabolite estimates and corresponding Cramer-Rao lower bounds for the mean spectra of the control and disease mice using the MM and splines to fit the background, (b) Metabolite estimates with standard deviation among all signals in each group.

Conclusion

Results obtained in this study show that there are no significant differences between control and APP mice visible in the available MRS spectra. The background signal could probably affect the expected observable small variations in metabolites such as NAA [4,5] in the APP group. Additionally, the age differences of APP mice might also influence the variability of metabolites compared to the control mice. On the other hand, the use of a measured macromolecular background has not been used in this kind of studies and we observed that good fits are obtained when the MMs are used, especially when computed together with splines, which compensates for the extra background contributions. In the absence of measured MM signals, we proved that allowing a flexible spline model in AQSES can compensate for the missing macromolecular components. No significant differences were observed when quantifying using the simulated basis set. Finally, variability in quantification results showed that detection of Alzheimer disease using ^{1}H MRS in the hippocampus is not providing the class separation expected between the used control and APP mice.

References: [1] J-B. Poullet, et al., *NMR Biomed., 20(5):493 – 504, 2007*. [2] D. Stefan, et al., Meas. Sci.Technol., 20(10):104035(9pp), 2009. [3] S.W. Provencher, NMR Biomed., 14(4):260–264, 2001. [4] M. Marjanska, et al., PNAS, 33 (102):11906-11910, 2005. [5] R. Dixon, et al., Brain, 125, 2332-2341, 2002. [6] Moechars et al., J Biol Chem, 274: 6483-6492, 1999.