Separate Signal Detection of Glutamate and Glutamine in the Rat Brain *in Vivo* at 4.7 T Using Optimized ^{1}H-CT-PRESS With Effective Homonuclear Decoupling

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Introduction:
Besides the signal-to-noise ratio (SNR) per constant total measuring time T_{meas}, the quality of signal separation and peak assignment is of central importance for the value of *in vivo* ^{1}H magnetic resonance spectroscopy (MRS) or spectroscopic imaging (SI). In standard MRS/SI with 1D spectral resolution it is difficult to separate the signals of the amino acids glutamate (Glu) and glutamine (Gln), which are a vital part of neurotransmission and metabolism in the brain. This problem is mainly due to the small chemical shift differences, rather large J coupling constants and/or the superposition with other resonances (e.g., of N-acetylaspartate (NAA)) (1-3). Therefore, based on 1D MRS/SI, Glu and Gln signals can only be extracted by data postprocessing using prior knowledge about the J coupling patterns and the chemical shifts. Alternatively, improved signal differentiation can be achieved by methods of 2D MRS (1). In the following it is shown that Glu and Gln signals can be separated in the rat brain *in vivo* using optimized CT-PRESS (4), allowing to detect spectra with effective homonuclear decoupling and high SNR.

Experimental:
Hardware: All experiments were carried out on a 4.7T/40cm Bruker Biospec system using a 98mm diameter saddle-type resonator for RF excitation. For signal reception the same resonator (phantom measurements) or an 18mm surface coil (*in vivo* measurements) was used. Phantom studies were carried out on 50 or 100 mM solutions of Glu, Gln or other compunds of interest. *In vivo* measurements were performed on the brain of healthy male Wistar rats which were anaesthetized using 0.8-1.5% halothane in 7:3 N_2O/O_2.
Measuring Method: The CT-PRESS sequence was modified to give optimal results for the detection of Glu and Gln:

[CHESS]-[90°(z)-180°(x)--180°(y)-]-t_1/2-180°-t_2(acquis.).

Thus CT-PRESS is similar to FOCSY-J (5): However, CT-PRESS uses experimental parameters that are optimal for the detection of projection spectra onto the f_1 axis giving effective homonuclear decoupling. Furthermore, there are differences in the data processing. Thus the apodization functions are optimized for metabolites of interest dependent on the J coupling and T_2, and shifts of the raw data make a 45° tilt in the 2D spectrum dispensable. The experimental parameters were as follows: water suppression by three consecutive 15 ms Gaussian RF pulses; TE of the PRESS module: 16 ms; F_2=4006 Hz; 1K complex data points sampled in t_2; $\Delta t_1/2$=4 ms corresponding to F_1=125 Hz; 22-28 t_1 steps with the central t_1 value corresponding to a TE of 136 ms, which was empirically determined to give the best results for Glu and Gln; TR=2.0 s; 4 accumulations, voxel size: 4*4*4 or 5*5*5 mm^3.
Data Processing: CT-PRESS spectra were calculated as projection spectra onto the f_1 axis considering the wrap around caused by the large Δt_1. The integration width around the diagonal of the 2D spectrum was +/- 14 Hz. The raw data were apodized in t_1 and t_2 with generalized sine-bell functions.

Results and Discussion:
Figure 1 shows the CT-PRESS spectrum of a solution of Glu+Gln (each 50 mM). The effective homonuclear decoupling allows a clear separation between the γ-CH_2 of Gln (2.46 ppm) and the γ-CH_2 of Glu (2.36 ppm). The β-CH signal of Gln (2.14 ppm) is, although not entirely, separated from the β'-CH (2.10 ppm) and β-CH (2.05 ppm) signal of Glu. The α-CH signals of Glu (3.76 ppm) and Gln (3.78 ppm) are unresolved.
In Fig.2 a CT-PRESS spectrum of the healthy rat brain is depicted. Besides the predominant singlet signals of NAA (2.02 ppm), total creatine (tCr) and choline containing compounds (Cho), the coupled resonances of myo-inositol (Ins), taurine (Tau), and NAA are well resolved. The α-CH signals of Glu+Gln (Glx) appear at 3.77 ppm. The γ-CH_2 of Glu at 2.36 ppm is detected with high SNR and narrow linewidth, so that the superposition with the γ-CH_2 signal of Gln (2.46 ppm) or the α-CH_2 of GABA (2.31 ppm, (1)) is negligible. Since the γ-CH_2 of Gln (2.46 ppm) is superimposed by NAA resonances, this signal is not appropriate for detecting Glu. However, the β-CH signal of Gln at 2.14 ppm is sufficiently well resolved from the β'-CH (2.10 ppm) and β-CH (2.05 ppm) signal of Glu and the intense CH_3 signal of NAA (2.02 ppm). Thus the β-CH signal can be used to detect Gln in *in vivo* ^{1}H spectra with effective homonuclear decoupling.

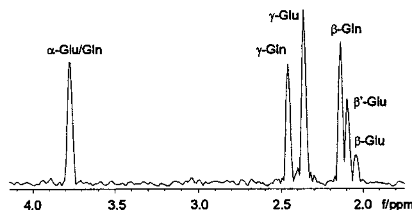

Fig.1: CT-PRESS spectrum of a model solution of Glu + Gln (each 50 mM).

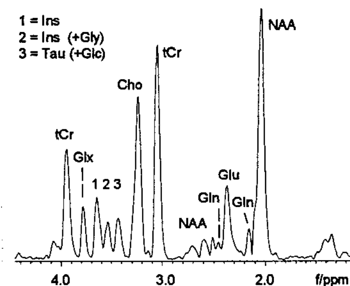

Fig.2: CT-PRESS spectrum measured on the healthy rat brain *in vivo* (5*5*5 mm^3, 22 t_1 steps, T_{meas}=3 min).

Conclusion:
Acquiring CT-PRESS spectra with effective homonuclear decoupling is an efficient way to detect signals of Glu and Gln separately using the γ-CH_2 of Glu (2.36 ppm) and the β-CH signal of Gln (2.14 ppm). Compared with earlier CT-PRESS measurements, improved spectral resolution was achieved because an enlarged and optimized t_1 range was used to detect Glu and Gln. Since both the the SNR and the reproducibility of the spectra are high, this sequence will allow to detect changes of Glu and/or Gln in pathologies and to perform parametric studies of these metabolite signals (e.g., T_1, diffusion behaviour, magnetization transfer effects). Considering the short minimum total measuring time (< 1 min) an extension to MRSI will be possible, either based on Hadamard encoding or using fast MRSI methods with oscillating gradients during signal acquisition.

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