In vivo metabolite differences between the basal ganglia and cerebellum of the rat brain detected with proton MRS at 3 T

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Introduction

In vivo magnetic resonance spectroscopy (MRS) permits non-invasive longitudinal tracking of brain chemistry changes that can accompany aging, alcohol dependence, and experimental manipulations modeling such conditions.[1,2] As 3 T human scanners have become widely available, animal research at the same field strength with the same protocols should facilitate preclinical to clinical translational research. Constant Time PRESS (CT-PRESS)[3] has been introduced as a method to detect coupled resonances with high signal-to-noise ratio by using effective homonuclear decoupling. Here, we tested the feasibility of using CT-PRESS optimized for the detection of glutamate (Glu)[4] in the rat to dissociate the biochemical profile of two brain regions affected by alcohol dependence, the basal ganglia and cerebellum. Our aim was to establish baseline levels of principal metabolites detectable with proton MRS in different brain regions using tissue water as an internal reference.

Methods

All measurements were performed on a GE 3 T MR scanner equipped with self-shielded gradients (40 mT/m, 150 mT/m/ms). A quadrature birdcage coil (∅ = 44 mm) was used for both RF excitation and signal reception. A group of six healthy male adult Wistar rats (435-523 g body weight) were scanned in sessions of ~1.5 h each. Anesthesia was provided by 2-3.5% isoflurane in oxygen (~1.5 l/min).

The implemented CT-PRESS sequence consisted of a modified PRESS module in which the position of the last refocusing pulse was shifted to encode the chemical shift (CS) in the second time dimension (t_1). The average echo time (TE) of the sequence was 139 ms optimized for the detection of Glu (129 CS encoding steps, $\Delta t_1/2$ = 0.8 ms, TR = 2 s). CT-PRESS was preceded by a 3-pulse CHESS sequence for water suppression and an outer-volume suppression module using very selective saturation pulses. With 6 averages, the acquisition time per spectrum was ~26 min. An acquisition without water suppression was carried out with 17 CS encoding steps ($\Delta t_1/2$ = 6.4 ms, 8-fold undersampling) and 2 averages to measure the tissue water content which was used to normalize the signal intensities of the metabolite data. For each rat, spectra were acquired from two voxels: the first contained mostly the basal ganglia, the second the cerebellum. The voxel dimensions were 8.6 mm (L/R), 5 mm (A/P), and 4.8 mm (S/I). Typical voxel locations are shown in Fig. 1.

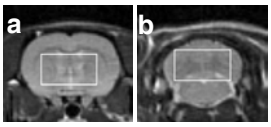

Fig. 1: Coronal FSE images displaying voxel position in the basal ganglia (a) and the cerebellum (b).

Apodization of the water suppressed data comprised multiplication with sine-bell functions in both time dimensions and zero-filling up to 4K×1K data points. A t_1-dependent shift was applied in t_2 correcting the different start of data acquisition. After 2D FFT, effectively decoupled 1D CT-PRESS spectra were obtained by integrating the signal along f_2 within a ±10 Hz interval around the spectral diagonal. Metabolite signals in the 1D spectra were evaluated by peak integration with an interval of ±6 Hz. The amount of tissue water in a voxel was estimated from the data set acquired without water suppression. For each TE step, only the time domain data from each TE onward was used. After FFT along t_2, water spectra were evaluated by peak integration with an interval of ±50 Hz. The amount of cerebral spinal fluid and tissue water was estimated by fitting the data to a bi-exponential model. In addition to use of water referencing, metabolite intensities were calculated relative to the methyl signal of total creatine (tCr), a quantitation approach commonly used in human clinical studies.

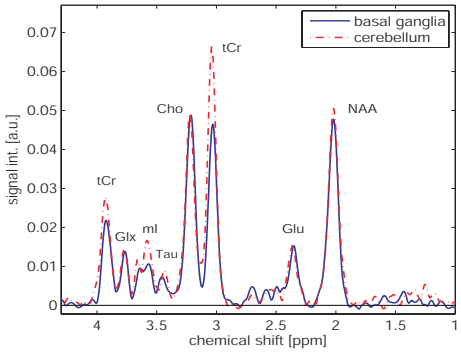

Fig. 2: Representative CT-PRESS spectra from voxels in the basal ganglia (solid blue) and cerebellum (dashed red) of a single rat. The spectra were normalized to the amount of tissue water.

Results and Discussion

Representative CT-PRESS spectra from the two brain regions in a single rat are shown in Fig. 2. The quality of the spectra allowed evaluation of signals from N-acetyl-aspartate (NAA), tCr, choline containing compounds (Cho), Glu, Glu+glutamine (Glx), *myo*-inositol (mI), and taurine (Tau). Mean signal intensities and standard deviations are given in Table 1. Using the amount of tissue water as an internal reference, both tCr (paired t-test, p = 0.0031) and mI (p = 0.0049) were lower in the basal ganglia than cerebellum. This agrees with an MRS study at 9.4 T of mouse brain in vivo which reports some of the largest differences in absolute concentrations between the cerebellum and the striatum for tCr and mI.[5] In our study, the remaining metabolites showed no statistically significant differences between the two regions. Not surprisingly, the pattern of regional metabolite estimates was notably different when intensities were expressed as ratios to tCr. Basal ganglia had higher values than cerebellum for NAA (p=0.0001), Glu (p=0.0002), Cho (p=0.0001), Tau (p=0.0053), and Glx (p=0.0011), comporting with previous reports using rat brain extracts[6] when expressed in ratios to tCr.

Higher levels of tCr in the cerebellum might imply a higher energy demand compared with the basal ganglia[7]. However, further interpretation of this finding would require studies using ^{31}P MRS to measure tissue concentrations of phosphorus metabolites involved in energy and membrane phospholipid metabolism. A possible explanation of the higher mI signals in the cerebellum compared with the basal ganglia may be that the phosphatidylinositol second messenger system, of which mI is a major constituent, is a more important signal transduction mechanism than previously recognized or that there are relatively more glia in the cerebellum than basal ganglia[8]. The latter interpretation is tempered by findings indicating that high levels of mI are also present in neurons.[9,10]

a)	basal ganglia	cerebellum
NAA	0.71 ± 0.05	0.75 ± 0.06
Glu	0.18 ± 0.02	0.17 ± 0.03
tCr *	0.69 ± 0.06	0.95 ± 0.06
Cho	0.76 ± 0.07	0.69 ± 0.04
Tau	0.12 ± 0.02	0.11 ± 0.01
mI *	0.16 ± 0.02	0.23 ± 0.02
Glx	0.19 ± 0.01	0.18 ± 0.02

b)	basal ganglia	cerebellum
NAA/tCr *	1.03 ± 0.03	0.79 ± 0.03
Glu/tCr *	0.27 ± 0.07	0.18 ± 0.02
Cho/tCr *	1.11 ± 0.06	0.74 ± 0.07
Tau/tCr *	0.17 ± 0.02	0.12 ± 0.01
mI/tCr	0.24 ± 0.05	0.25 ± 0.01
Glx/tCr *	0.28 ± 0.08	0.19 ± 0.02

Table 1: Mean intensities and standard deviations of metabolite signals in the basal ganglia and cerebellum: (a) relative to tissue water and (b) tCr. * ($p \leq 0.0053$)

Conclusion

This study demonstrates the feasibility of using in vivo CT-PRESS to quantitate differences in metabolite signals between brain regions of the rat. CT-PRESS provides the enhanced signal separation necessary to reliably measure J-coupled resonances, including Glu and mI, in the rat brain at 3 T. Our findings, which indicate variation of tCr across brain regions, emphasize the benefit to in vivo MRS of incorporating methods to establish baseline metabolite signals relative to water. Such advances should contribute to validating an animal model of chronic alcoholism, a disease which induces glutamatergic neurotransmitter system changes in striatal regions and structural and biochemical changes to cerebellar regions.

Support

AA05965, AA12388, AA13521 (INIA).

References

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