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Highly Resolved Two-dimensional ^1H Spectroscopy of the Human Brain using ISIS CT-PRESS with Resolution Enhancement

Hidehiro WATANABE*, Nobuhiro TAKAYA, and Fumiyuki MITSUMORI

Center for Environmental Measurement and Analysis, National Institutes for Environmental Studies

16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

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In constant time (CT) point-resolved spectroscopy (PRESS), echo centers shift with the fast decay of short T_2^* on two-dimensional (2D) time domain (TD) data under inhomogeneous B_0 field like *in vivo* conditions. Though ^1H decoupling along the F_1 direction is a feature of this method, the tilted and broadened peak pattern on the F_1 - F_2 plane after reconstruction causes the peaks to overlap.

To enhance resolution to achieve highly resolved 2D CT-PRESS spectra in the human brain, we propose a 2-part window function that comprises an enhancement part for shifting echoes with fast decay and a conventional part, such as Lorentzian, Gaussian, or sine-bell function.

We obtained 2D spectra from human brains at 4.7T. The 3 diagonal peaks of C4H of glutamate (Glu C4H) at 2.35 ppm, C2H of γ -amino butyric acid (GABA C2H) at 2.28 ppm, and C4H of glutamine (Gln C4H) at 2.44 ppm—overlapped on the spectra processed with the conventional window but clearly resolved on the spectra using the proposed enhancement window. The signal-to-noise ratio per unit measurement time of Glu C4H on a CT-PRESS spectrum of the human brain was 1.7 times higher than that on a spectrum obtained by CT-correlation spectroscopic (COSY).

In conclusion, 2D CT-PRESS spectra processed with the proposed window function to enhance resolution can resolve peaks of coupled ^1H spins with higher accuracy and sensitivity.

Keywords: CT-PRESS, GABA, glutamate, human brain, *in vivo*

Introduction

In the human brain, glutamate (Glu) is a major excitatory neurotransmitter, and γ -amino butyric acid (GABA) is a major inhibitory neurotransmitter. Glutamine (Gln) is a precursor and storage form of Glu that is predominantly synthesized in astrocytes and plays an important role in the Glu and Gln cycle in the brain. Because these metabolites have complex spin systems as a result of ^1H - ^1H homonuclear couplings (J_{HH}) with small chemical shift differences, peaks overlap on conventional one-dimensional (1D) ^1H spectra of the human brain, even at 3T while it has shown that Glu and Gln are resolved at 7T, GABA is still overlapped on resonance of Glu.¹

Constant time (CT) two-dimensional (2D) meth-

ods demonstrate good peak resolution through ^1H decoupling along the F_1 direction. We have reported resolution of the 3 diagonal peaks of the C4 protons of Glu (Glu C4H) and Gln (Gln C4H) and C2 proton of GABA (GABA C2H) at about 2.4 ppm on localized 2D CT-correlation spectroscopy (COSY) spectra in the human brain at 4.7T.² Those results indicate that these peaks can also be resolved on spectra obtained by CT-point-resolved spectroscopy (PRESS),^{3,4} where only diagonal peaks appear. CT-PRESS method is a spin echo type of CT techniques and higher sensitivity is expected. However, worse resolution of overlapped peaks than expected has been reported on *in vivo* spectra acquired using CT-PRESS.^{3,4}

In this work, we demonstrate the resolution of the 3 peaks of Glu, GABA, and Gln on spectra of the human brain obtained by CT-PRESS processed using our proposed window function for resolution enhancement. We also compare sensitivity between

*Corresponding author, Phone: +81-29-850-2138, Fax: +81-29-850-2880, E-mail: idewata@nies.go.jp

spectra acquired by CT-PRESS and by CT-COSY.

Materials and Methods

ISIS CT-PRESS sequence⁵

We developed the image-selected *in vivo* spectroscopy (ISIS) version of CT-PRESS (Fig. 1), in which water suppression and outer volume suppression are followed by a module for localization: ISIS pulse -90° slice pulse $-1/2 \cdot TE1 - 180^\circ$ non-slice pulse $-1/2 \cdot (TE1 + TE2) + (n-1)\Delta t_1/2 - 180^\circ$ slice pulse $- \{ \text{data acquisition } (t_2\text{-direction}) \}$. Each FID

Fig. 1. An ISIS CT-PRESS sequence. A 1D ISIS method is used instead of a 180° pulse in the CT-PRESS module to minimize chemical shift displacement errors and improve the slice profile. In a CT-PRESS module in which a 180° slice pulse is shifted by $\Delta t_1/2$ every t_1 step, the generated FID accumulates along the t_2 immediately after the crusher gradient pulse. The ^1H chemical shift (δ_H) is refocused by 180° pulses in the CT-PRESS module; J_{HH} is not. Then, J_{HH} evolution at any t_1 is the same amount at the constant time delay after the 90° pulse. The t_1 -dependent shift is applied to the accumulated FIDs to attain the constant time condition after whole t_1 steps. δ_{HH} evolves along both the t_1 and t_2 directions, and J_{HH} , along only t_2 . Then, ^1H decoupled spectra along F_1 are reconstructed.

is acquired along the t_2 direction at every t_1 increment where the position of the 180° slice pulse is shifted by $\Delta t_1/2$. After the whole scan, the 2D time domain (TD) data, $S(t_1, t_2)$, is collected, and a 2D spectrum is generated after 2D reconstruction. Though the evolution of J_{HH} is constant at every t_1 increment at the constant time delay, T_{ct} , after 90° slice pulse, chemical shift, δ_H , evolves. Then, J_{HH} is decoupled along the F_1 direction on the 2D spectrum.

One direction is localized by the ISIS module instead of a 180° slice pulse in the following CT-PRESS module. A radiofrequency (RF) pulse with wide band width is applicable because the ISIS module is applied before generation of transversal magnetization. This minimizes chemical shift displacement errors to achieve a better slice profile and shortens echo time by 180° non-slice pulse of short duration. Data acquisition was started immediately after crusher gradient for the 180° slice pulse along the z -direction. After signal accumulations, a t_1 -dependent shift was applied along t_2 to attain the constant time condition.⁴

A window function of resolution enhancement for shifted echoes

On 2D TD data of CT-PRESS, the spin echo center is shifted by Δt_1 along the t_1 direction by the 180° slice pulse the position of which is shifted by $\Delta t_1/2$ (Fig. 2a). While this shifted configuration on TD data does not affect peak forms on reconstructed spectra under the long T_2^* condition of homogeneous B_0 field, peaks are tilted and broadened on the F_1 - F_2 plane of spectra under the short T_2^* condition of inhomogeneous B_0 field like *in vivo* spectra, worsening peak resolution (see Fig. 3d in Results).

Figures 2b and 2c show schematics of our proposed window to overcome this problem. The signal intensity of the spin echo is described as

Fig. 2. Schematics of 2D time domain (TD) data of constant time point-resolved spectroscopy (CT-PRESS) signals (a) and the proposed window function for resolution enhancement that consists of an enhancement part (b) and a conventional apodization part (c).

Fig. 3. ISIS constant time point-resolved spectroscopy (CT-PRESS) spectra of the brain phantom including (a) and excluding γ -amino butyric acid (GABA) (b). Spectra calculated by GAMMA (6) are also shown under the conditions of homogeneous (c) and inhomogeneous (d) B_0 . Peaks having positive intensities are displayed in a phase-sensitive mode on all spectra.

$e^{-|t-TE|/T_2^*}$, in which $|t-TE|$ denotes the absolute of time from the echo center at echo time, TE. After n FIDs are accumulated, TE is $TE1 + TE2 + t_1$, where $t_1 = (n-1)\Delta t_1$. Whereas nuclear magnetic resonance (NMR) signal decreases by T_2 relaxation along the indirect direction of t_1 , it decreases by T_2^* along the direct direction of t_2 . Then, the signal intensity of 2D TD data obtained by CT-PRESS is expressed as:

$$e^{-\frac{TE}{T_2}} e^{-\frac{|t_2-TE|}{T_2^*}} = e^{-\frac{(TE1+TE2+t_1)}{T_2}} e^{-\frac{|t_2-(TE1+TE2+t_1)|}{T_2^*}}.$$

In the proposed window, each FID on 2D TD data is multiplied with $e^{\alpha|t_2-(TE1+TE2+t_1)|}$ to flatten the shape formed by the shifting echo center (Fig. 2b). Next, 2D Lorentzian, Gaussian, or sine-bell function is applied to increase the signal-to-noise ratio (SNR) (Fig. 2c). Although a condition of $\alpha = 1/T_2^*$ is best to resolve enhancement, the increasing noise decreases the SNR. As a compromise, weaker enhancement with lower values of α should be used.

Experimentation

We performed all measurements using a 4.7T whole-body NMR spectrometer (Agilent, Palo-Alto, CA, USA) with a gradient system of 35 mT/m maximum gradient strength with a rise time of 350 μs and equipped with first- and second-order shim coils. We used a volume transverse electromagnetic (TEM) coil of 300-mm diameter for transmission and reception. The internal review board of the National Institute for Environmental Studies approved the protocol, and we obtained informed consent from volunteers before conducting measurements.

We first performed a phantom study, filling each of two 200-mL bottles with a different mixture of brain metabolites. One contained 10 mM N-acetyl aspartate (NAA), 8 mM creatine (Cr), 9 mM Glu, 3 mM Gln, and 2 mM GABA, and the other contained the same reagents except GABA. We placed the first bottle in a water bath containing 0.9% dissolved sodium chloride (NaCl) to mimic an *in vivo* load and acquired ISIS CT-PRESS signals within a

voxel in the bottle and then exchanged the bottles and acquired ISIS CT-PRESS signals for the second bottle.

We obtained spectra after reconstruction processed with a conventional window: Gaussian of 3 Hz along both t_1 and t_2 directions and Lorentzian of 3 Hz along the t_1 direction and of one Hz along t_2 . To assign peaks, spectra containing NAA, Cr, Glu, GABA, and Gln were calculated by the NMR simulation software of general approach to magnetic resonance mathematical analysis (GAMMA, 6) using published values of chemical shifts and ^1H couplings.⁷

In volunteer studies, we selected a volume of interest (VOI) of $30 \times 30 \times 30 \text{ mm}^3$ in a parieto-occipital region on a scout image. First, we achieved a line width of 10 Hz on the ^1H spectrum by fast automatic shimming technique by mapping along projections (FASTMAP). This width is equivalent to $T_2^* \sim 30 \text{ ms}$. After adjusting the RF power, we acquired ISIS CT-PRESS signals with measurement time of 20 min. After applying the t_1 -dependent shift along the t_2 to the acquired ISIS CT-PRESS signals,⁴ the proposed enhancement window was processed. Although α should be based on the T_2^* of peaks in metabolites, it is difficult to determine the T_2^* of J_{HH} coupled peaks, such as Glu C4H and GABA C2H. We then set the enhancement factor, α , to 5 Hz to discriminate the peaks of Glu C4H and GABA C2H on a reconstructed spectrum. We examine the determination of α in the Discussion. In the conventional window part, we processed Gaussian of 10 Hz and Lorentzian of 3 Hz along both the t_1 and t_2 directions. We also ob-

tained conventional spectra without resolution enhancement for comparison. Positive intensity peaks were displayed in a phase sensitive mode on all spectra. Zero-order and linear phases were obtained by 3 singlet peaks of NAA at 2 ppm, Cr at 3 ppm, and 3.9 ppm. TE1 was 15 ms and constant time delay, T_{ct} , was 124 ms in all measurements. TE2 was 14 ms in phantom experiments and 17 ms in volunteer measurements. Spectral width along F_1 was one kHz and along F_2 , 2 kHz. The number of t_1 increments, n_1 , was 150. Relaxation delays were 3 seconds for phantom experiments and 4 seconds for volunteer measurements.

Results

Figure 3a shows the CT-PRESS spectrum of the brain phantom including GABA and 3b, the spectrum excluding GABA. Peak resolution was improved by ^1H decoupling along the F_1 direction on both spectra. The 3 diagonal peaks at 2.28, 2.35, and 2.44 ppm were resolved on the spectrum including GABA (Fig. 3a), but the resonance at 2.28 ppm was absent from the spectrum excluding GABA (Fig. 3b). Three diagonal peaks also appeared on a simulated spectrum calculated by GAMMA (Fig. 3c). From these results, we assigned the peak at 2.28 to GABA C2H, 2.35 ppm to Glu C4H, and 2.34 ppm to Gln C4H. Figure 3d shows a simulated spectrum calculated under $T_2^* = 30 \text{ ms}$. In contrast to clearly resolved peaks on a simulated spectrum under $T_2^* = 300 \text{ ms}$ (Fig. 3c), these peaks overlapped in inhomogeneous case.

Figure 4 shows conventional (a) and resolution-

Fig. 4. ISIS constant time point-resolved spectroscopy (CT-PRESS) spectra obtained from a parieto-occipital region of 27 mL in a human brain, applied with Gaussian and Lorentzian apodization functions (a) and with the proposed resolution enhancement function (b). Positive peaks are displayed in a phase-sensitive mode.

enhanced (b) spectra obtained from a human brain. On the conventional spectrum, the 3 peaks of Glu C4H, GABA C2H, and Gln C4H tilted and overlapped. Especially, Glu and GABA were not resolved. This pattern is similar to the simulated spectrum under short T_2^* condition (Fig. 3d). In contrast, these 3 peaks were resolved on the enhanced spectrum. Although the peak of Glu C4H remained somewhat tilted because of weaker enhancement, that of GABA C2H was well resolved compared to the conventional spectrum. On the enhanced spectrum, artifacts due to an NAA singlet arose, but they did not interfere with the metabolite peaks of Glu C4H, GABA C2H, and Gln C4H.

We simulated the spectra of Glu, GABA, and Gln at 3, 4.7, 7, and 9.4 T using published values of chemical shifts and J_{HH} ⁷ to compare peak resolution between CT-PRESS and the 1D method (Fig. 5). We calculated the FID signal generated directly after one pulse by GAMMA⁶ under *in vivo* conditions at line widths of 5 and 10 Hz.¹ Ivan examined typical line widths of 5.5 Hz for very well shimmed volumes in the human brain at 4 T and a typical value of 9.5 Hz at 7 T.¹ This means that consideration under the both conditions of 5 and 10 Hz is enough at various B_0 fields. Whereas the 3 diagonal peaks of Glu C4H, GABA C2H, and Gln C4H were resolved in the range between 2.1 ppm and 2.6 ppm on a CT-PRESS spectrum of the human brain (Fig. 4b), they overlapped in the same range in the simulated 1D spectra at 4.7 T at both 5 and 10 Hz. Though peaks of Glu C4H and Gln C4H were resolved over 7 T, the peak of GABA C2H still overlapped and resolved only at 5 Hz at 9.4 T.

Discussion

In CT-COSY, another CT method, two 90° pulses generate half of full magnetization to yield diagonal peaks. This method also allows measurement of the connectivity between spins as cross peaks, which are usually distant from the crowded diagonal peaks. While the cross peaks are more suitable than diagonal peaks for peak resolution, the diagonal peaks have higher signal intensities than the cross peaks.² We could demonstrate the resolution of even diagonal peaks by ^1H decoupling along the F_1 direction on the CT-COSY spectra of the human brain at 4.7 T,² so we expected good peak resolution and high sensitivity of the diagonal peaks on 2D CT spectra.

Higher sensitivity is expected in CT-PRESS than CT-COSY because full magnetization is tipped to the transversal plane in the spin-echo-type CT-PRESS. However, tilted and broadened peak patterns cause peaks to overlap on the F_1 - F_2 plane of *in vivo* CT-PRESS spectra. This peak pattern arises from short T_2^* by inhomogeneous B_0 field like *in vivo* condition. To overcome this problem, we have proposed a window function for resolution enhancement; using the proposed window, we demonstrated the clear resolution of the 3 diagonal peaks of Glu C4H, GABA C2H, and Gln C4H on the human brain spectra at 4.7 T.

To discriminate the peaks of Glu C4H and GABA C2H on a reconstructed spectrum, we determined the value of $\alpha = 5$ Hz. In the human brain, the line width of the water ^1H spectrum was 10 Hz after shimming and the T_2 of water ^1H is around 60

Fig. 5. Simulated spectra of glutamate (Glu), γ -amino butyric acid (GABA), and glutamine (Gln) at various B_0 fields, calculated by GAMMA(6) at line width of 5 Hz (a) or 10 Hz (b).

ms at 4.7T.⁸ The magnetic field inhomogeneity, ΔH , in the voxel is around 5 Hz from the equation:

$$\frac{1}{\pi T_2^*} = \frac{1}{\pi T_2} + \Delta H.$$

Because the T_2 of Glu C4H is around 120 ms,⁹ the line width of Glu C4H is around 7.7 Hz, which is a little narrower than the 10-Hz line width of water ^1H . Therefore, the value of α should be practically set to the narrower value of the linewidth of water ^1H in the human brain. The utilized value of $\alpha=5$ Hz meets this condition.

To evaluate sensitivity improvement in CT-PRESS, we compared the SNR of Glu C4H on the spectra of both CT-COSY and CT-PRESS. The SNR per unit measurement time ($\text{SNR}_{\text{unit-time}}$) was 2.24 times higher on the CT-PRESS spectrum of the brain phantom than the CT-COSY spectrum. This value is a little higher than the theoretical value of 2, perhaps because of the differences in Gaussian and Lorentzian window functions between CT-PRESS and CT-COSY. In the human brain, $\text{SNR}_{\text{unit-time}}$ also improved by a factor of 1.7 on the spectrum processed with the proposed window. While the proposed window was not used on the phantom spectrum, it was used on the human brain spectrum. Although this window of resolution enhancement improves peak resolution, it also enhances noise on the spectra. This tradeoff between improved peak resolution and increased noise may be responsible for the difference in improvement factors between the phantom and human brain.

Procedures similar to those on 1D spectra can be used to quantitate peaks of metabolites on *in vivo* 2D spectra^{9,10}; peak volumes on 2D spectra should be calculated in the same manner as peak areas in 1D spectra and corrections on T_1 and T_2 relaxations are also required. Internal water reference methods can also be applied for absolute quantitation. Effects of T_1 relaxations can be decreased using a slightly prolonged relaxation delay of $\sim 3T_1$. T_2 can be obtained by measuring several spectra with varied T_{ct} . Because a metabolite has several peaks with different T_2 values, all peaks in a metabolite, such as GABA C2H, C3H, and C4H, cannot be corrected by the same T_2 . Instead, one resolved peak, such as that of GABA C2H, should be utilized for quantitation by T_2 correction. The shared TD data method can be used to shorten the time needed for T_2 measurements.¹⁰

T_2 correction is not required for quantitation of ^1H spectra with a short TE achievable by the 1D method. This method allows shorter measurement time for the ^1H spectra than with the CT-PRESS

method. Then, the 1D method should be used for the peaks of singlets of NAA and Cr. Despite problems of peak overlap for metabolites having complex spin systems caused by J_{HH} , such as Glu, GABA, and Gln, the shorter measurement time with the 1D method recommends its use as first choice. However, the good peak resolution using the CT-PRESS method demonstrates its superiority for more accurate quantitation of those metabolites.

Conclusion

Although 2D CT-PRESS offers ^1H decoupling along the F_1 direction, tilted and broadened peak patterns cause peaks to overlap *in vivo* when T_2^* is shortened by inhomogeneous B_0 fields. We demonstrated resolution of the 3 diagonal peaks of Glu C4H, GABA C2H, and Gln C4H with higher sensitivity on human brain spectra at 4.7T processed with our proposed enhancement window. In conclusion, 2D CT-PRESS spectra processed with the proposed resolution enhancement window can resolve peaks of coupled ^1H spins with high accuracy and sensitivity.

References

1. Tkáč I, Andersen P, Adriany G, Merkle H, Uğurbil K, Gruetter R. *In vivo* ^1H NMR spectroscopy of the human brain at 7T. *Magn Reson Med* 2001; 46:451–456.
2. Watanabe H, Takaya N, Mitsumori F. Simultaneous observation of glutamate, γ -aminobutyric acid, and glutamine in human brain at 4.7T using localized two-dimensional constant-time correlation spectroscopy. *NMR Biomed* 2008; 21:518–526.
3. Dreher W, Leibfritz D. Detection of homonuclear decoupled *in vivo* proton NMR spectra using constant time chemical shift encoding: CT-PRESS. *Magn Reson Imaging* 1999; 17:141–150.
4. Mayer D, Spielman DM. Detection of glutamate in the human brain at 3T using optimized constant time point resolved spectroscopy. *Magn Reson Med* 2005; 54:439–442.
5. Watanabe H, Takaya N, Mitsumori F. Sensitivity improvements in peak detection of glutamate, GABA and glutamine in the human brain using ISIS CT-PRESS at 4.7T. *Proc Intl Soc Mag Reson Med* 2008; 16:1610.
6. Smith SA, Levante TO, Meier BH, Ernst RR. Computer simulations in magnetic resonance. an object-oriented programming approach. *J Magn Reson Ser A* 1994; 106:75–105.
7. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for

- brain metabolites. NMR Biomed 2000; 13:129–153.
8. Mitsumori F, Watanabe H, Takaya N, Garwood M. Apparent transverse relaxation rate in human brain varies linearly with tissue iron concentration at 4.7T. Magn Reson Med 2007; 58:1054–1060.
 9. Watanabe H, Takaya N, Mitsumori F. Quantitation of glutamate and GABA in the human brain *in vivo* using localized 2D constant time COSY at 4.7T. Proc Intl Soc Mag Reson Med 2007; 15:201.
 10. Watanabe H, Takaya N, Mitsumori F. Fast T_2 correction and quantitation method on highly resolved 2D constant time ^1H spectra in human brain using 2D FT of shared time domain data. Proc Intl Soc Mag Reson Med 2009; 17:2388.
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