Published in final edited form as:

Magn Reson Med. 2010 November ; 64(5): 1247–1251. doi:10.1002/mrm.22536.

Measurement of N-acetylaspartylglutamate in the Human Frontal Brain by ^1H MRS at 7T

Changho Choi^{1,*}, Subroto Ghose², Jinsoo Uh¹, Aditya Patel¹, Ivan E. Dimitrov^{1,3}, Hanzhang Lu¹, Deborah Douglas¹, and Sandeep Ganji¹

¹ Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, USA

² Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA

³ Philips Medical Systems, Cleveland, OH, USA

Abstract

N-acetylaspartylglutamate (NAAG) in human brain has been measured with difference editing at 7T. The CH_2 proton resonances (~ 2.5 ppm) of the aspartyl groups of NAAG and NAA (N-acetylaspartate) were difference edited (MEGA) using 20-ms Gaussian radio-frequency pulses for selective 180° rotations of the coupling partners at 4.61 and 4.38 ppm, respectively. The echo time of the editing sequence, 108 ms, was obtained in phantom tests. Single-voxel localized *in vivo* measurements were conducted in the medial prefrontal and right frontal cortices of five healthy volunteers. The gray and white matter fractions within the voxels were obtained from T_1 -weighted image segmentation. Using linear regression of the metabolite concentration vs. fractional white matter contents within the voxels, the NAAG-to-NAA concentration ratios in gray and white matter were estimated to be 0.13 and 0.28 by difference editing (95% confidence intervals 0.07 – 0.19 and 0.22 – 0.34), respectively, assuming identical relaxation effects between the metabolites.

Keywords

NAAG; NAA; difference editing; 7T; human frontal brain; gray matter; white matter

INTRODUCTION

Measurement of N-acetylaspartylglutamate (NAAG) in human brain by proton MR spectroscopy is challenging due to its relatively low concentration and the overlap with intense signals of N-acetylaspartate (NAA) and glutamate (Glu). An approach of measuring NAAG is spectral fitting of the acetyl CH_3 singlet at 2.045 ppm (1), which is overlapped with the large NAA singlet at 2.010 ppm. Detection of this NAAG singlet is further complicated by the underlying multiplets of Glu, Gln (glutamine), GSH (glutathione) and the NAAG glutamate moiety at ~ 2 ppm. Due to these spectral complexities, good shimming is required for differentiating the NAAG and NAA singlets, as demonstrated in prior studies at 2T (2), 1.5T (3), and 7T (4). Alternatively, NAAG can be measured by means of J difference editing, as reported at 3T recently (5). The CH_2 resonances of the aspartyl groups overlap with each other, but the resonances of their coupling partners (CH protons) are quite distinct; *i.e.*, 4.61 and 4.38 ppm (6). This 0.23-ppm spectral distance may be utilized for selective detection of the NAAG and NAA CH_2 resonances by difference editing.

*Correspondence to: Changho Choi, PhD, Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA, Phone: 214-645-2805, FAX: 214-645-2885, changho.choi@utsouthwestern.edu.

High field MRS may benefit from the enhanced spectral resolution and increased thermal equilibrium magnetization. The resolution of coupled resonances is improved because the coupling strength which governs the overall linewidth of multiplets is independent of field strength B_0 . However, the spectral resolution between singlets may remain about the same with increasing B_0 when the lines get broader in proportion to B_0 . Given the difficulty with precise detection of the NAAG singlet, we have employed a single-voxel localized difference editing method for differentiating between the aspartate CH_2 resonances of NAAG and NAA at 7T. The regional difference of the metabolite levels was obtained using linear regression between the edited signals vs. white matter fractions within the voxels. Preliminary *in vivo* results from the human frontal brain are presented.

METHODS AND MATERIALS

Experiments were carried out on a whole-body 7.0 T scanner (Philips Medical Systems, Cleveland, OH, USA). A quadrature transmit volume head RF coil with a 16-channel phased-array receiver insert was used. The maximum available RF field intensity (B_1) for human brain was 15 μT . Slice selective RF pulses included an 8.8-ms amplitude/frequency-modulated 90° RF waveform (bandwidth (BW) = 4.7 kHz) and an 11.9-ms amplitude-modulated 180° RF waveform (BW = 1.4 kHz) (7).

Difference editing was employed for measurement of NAAG and NAA in human brain. The CH_2 proton resonances (~ 2.5 ppm) of the NAAG and NAA aspartyl groups were edited with selective 180° rotations of their coupling partner CH protons (at 4.61 and 4.38 ppm, respectively) within a J difference editing scheme (MEGA) (8)

90 - 180 - E180 - 180 - E180 - Acquisition

$\leftarrow \text{TE}_1 \rightarrow \text{TE}_3/2 \rightarrow \text{TE}_2 \rightarrow \text{TE}_3/2 \rightarrow$

where 90 and 180 denote slice-selective 90° and 180° RF pulses. A 20-ms editing 180° RF pulse (E180) (Gaussian envelope; truncated at 10%; BW = 57 Hz) was used to selectively excite the CH-proton resonance in subsans. Symmetric carriers of the E180 pulse were applied alternatively to minimize the contamination between NAAG and NAA (5,9). Namely, the E180 carrier was set at 4.61 and 4.15 ppm for NAAG editing and 4.38 and 4.84 ppm for NAA editing. The echo time of the editing sequence was optimized in phantoms. Two spherical phantoms (inner diameter = 6 cm) were prepared at pH = 7.2; one with NAAG 20 mM and Cr 16 mM, and the other with NAA 20 mM, creatine (Cr) 14 mM and Glu 18 mM. Difference-edited spectra of NAAG and NAA were obtained at TE = 103 – 147 ms, with identical TE_1 and TE_2 at 26 ms. Here, 26 ms TE_1 was the shortest possible for chosen RF and gradient pulses on the scanner. Phantom spectra were obtained from a $25 \times 25 \times 25$ mm³ voxel. The repetition time (TR) was 10 s ($> 5T_1$ for the phantom solutions).

In vivo tests of the NAAG editing were conducted on five healthy volunteers (3 female and 2 male; age 25–35 years). The protocol was approved by the UT Southwestern Institutional Review Board. Written informed consent was obtained prior to the scans. Following a survey image acquisition, sagittal T_1 -weighted images were obtained with a MP-RAGE sequence (TR/TE/TI = 2500/3.7/1300 ms; flip angle = 8° ; field of view = $240 \times 240 \times 150$ mm; 150 slices; slice thickness = 1.0 mm). ^1H -MRS data were acquired from two regions using a voxel size of $25 \times 30 \times 30$ mm³; medial prefrontal (gray matter dominant) and right frontal (white matter dominant). First and second-order shimming was carried out for volume of $50 \times 50 \times 50$ mm³ using FASTMAP (10). The linewidth (FWHM) of the water signal was ~ 14 Hz at TE = 108 ms. The mean FWHM of Cr CH_3 singlet at the same echo time was 11 ± 1 Hz for both prefrontal and right frontal. Data acquisition parameters included TR = 2.5 s, spectral width = 5 kHz, and sampling points = 4096. A 64-step phase cycling

scheme was constructed for the three slice-selective RF pulses (*i.e.*, four steps of each RF pulse). Following 6 dummy scans, free-induction decays (FID) were recorded in 64 and 16 blocks for NAAG and NAA editing runs, respectively, each block with 4 averages. The E180 carrier was switched between the two values in alternate 4-average scans. The frequency drift during the editing scan was minimized by acquiring a water signal from the selected volume using a small flip angle ($\sim 3^\circ$) and then re-setting the synthesizer frequency prior to each single scan. This was completed in ~ 12 ms with an internal tool of the Philips MR scanner. Using this method, the residual frequency drift during the ~ 11 -min NAAG editing scan was within ± 3 Hz (standard deviation < 0.5 Hz). The carriers of slice-selective RF pulses were set at 2.5 ppm. A four-pulse variable-flip-angle scheme was used for water suppression (11). Unsuppressed brain water signals were acquired with the same gradient schemes as those of water-suppressed editing acquisitions.

MRS data from the scanner were processed using an in-house Matlab program. The multi-block FIDs were corrected individually for eddy current artifacts using the unsuppressed brain water signal. Residual frequency drifts were corrected individually using the NAA singlet as a reference. Subspectra were averaged for each E180 carrier, phase corrected, and then subtracted. Spectra were apodized with a 2-Hz exponential function. The concentration ratio of NAAG and NAA was estimated from the peak area between 2.4 – 2.8 ppm, assuming identical relaxation effects between NAAG and NAA *in vivo*.

The compositions of gray matter (GM), white matter (WM), and cerebrospinal fluids (CSF) within the volume selected for the MRS scans were estimated from the T_1 -weighted images using Statistical Parametric Mapping software (SPM5). The probabilities of the three components were calculated for each pixel of the image. The probabilities of each component were then averaged to obtain the GM and WM fractions in the MRS voxel. To obtain the NAAG-to-NAA concentration ratios in GM and WM, the concentration ratios from the prefrontal and right frontal regions of the 5 subjects were fitted to a linear function of fractional white matter content. Prism 5 (GraphPad Software Inc.) was used for the linear regression.

RESULTS

Figure 1 presents phantom difference spectra of the aspartate moiety CH_2 resonances of NAAG as a function of TE for TE = 103 – 148 ms with 5 ms increments. The spectral pattern and signal amplitude of edited multiplet changed with TE. For TE₁ and TE₂ at 26 ms, the phantom spectra showed relatively large signal intensity at TE < 130 ms and the signal degraded with increasing TE. The data suggest an optimal TE of 108 ms for NAAG editing for a tentative T₂ of 130 ms, a mean value of published Cr and NAA singlet T₂'s for human brain at 7T (12). This TE is approximately equal to $1/J$, where J is the coupling strength (9.5 Hz) of the NAAG 2.5 and 4.61 ppm resonances.

Figure 2 shows phantom spectra of difference editing of NAAG and NAA at (TE₁, TE₂, TE₃) = (26, 26, 56) ms. For phantom-1, the subscan-A of NAAG editing produced two positive peaks at 2.5 and 2.7 ppm and, the subscan-A' gave a small peak at 2.5 ppm and a larger positive signal at 2.7 ppm, thereby leading to a major peak at 2.5 ppm in the difference spectrum, (A–A')/2. The peak area of the edited CH_2 signal was measured as 16% with respect to the NAAG singlet at 2.045 ppm of the subspectrum. For the NAA editing, the NAAG signals in subspectra were identical, resulting in a null signal in the difference spectrum. For phantom-2 that contained NAA and Glu, the signals were all canceled in a difference spectrum of NAAG editing, and only the NAA CH_2 resonances were detected in the NAA-edited difference spectrum. The peak area of the NAA CH_2 edited signal was 16% relative to the NAA singlet at 2.01 ppm. The editing yields of NAAG and NAA in terms of

peak area were measured to be equal. This result indicates that the *in vivo* NAAG-to-NAA concentration ratio can be obtained from their peak area ratio, assuming identical relaxation effects between the metabolites.

Figure 3 displays representative *in vivo* subspectra and difference spectra from the human prefrontal and right-frontal cortices, together with the voxel positioning. Subscans with E180 carrier at 4.61 and 4.15 ppm gave an NAAG edited signal in the difference spectrum, $(A-A')/2$. An NAA difference spectrum, $(B-B')/2$, was obtained from subtraction between the 4.38 and 4.84 ppm subspectra. The spectral patterns of the edited NAAG and NAA multiplets were in agreement with the phantom results. The concentration ratio between NAAG and NAA was obtained from the edited peak area ratio, calculated between 2.4 and 2.8 ppm, indicated by vertical lines in the figure. The mean signal-to-noise ratio (SNR) of edited NAAG peaks was 13 ± 3 and 16 ± 4 (mean $\pm$ SD, $n=5$) for prefrontal and right frontal, respectively, as measured from the peak amplitude with respect to the standard deviation of the noise levels in 0.5 – 1 ppm.

Figure 4 presents the NAAG-to-NAA concentration ratio, $[NAAG]/[NAA]$, as a function of the fractional white matter content for the prefrontal and right frontal data from the five subjects. A linear correlation of $[NAAG]/[NAA]$ vs. fractional white matter content was statistically significant ($p = 0.014$), with goodness of fit at 0.55. The concentration ratios in GM and WM were estimated from the intercepts of the linear regression line to the ordinate axes, assuming that the metabolite levels in CSF are negligible and relaxation effects are identical between NAAG and NAA. The concentration ratios in GM and WM were obtained 0.13 and 0.28 (95% confidence intervals 0.07 – 0.19 and 0.22 – 0.34), respectively.

DISCUSSION

The current paper reports NAAG and NAA levels in the human frontal brain as measured by difference editing. The result indicated that the NAAG-to-NAA concentration ratio is ~2-fold greater in WM than in GM for the human frontal brain. The NAAG and NAA measures of the current study are in good agreement with the prior 1H -MRS study by Pouwels and Frahm (2), which reported $[NAAG]/[NAA]$ of approximately 0.1 and 0.2 in the GM- and WM-dominant 8-12 ml volumes in the frontal brain, respectively, although the data may not be directly comparable because the present data were estimated for pure GM and WM. The NAAG-to-NAA concentration ratio appears to be higher in the WM-rich parietal and centrum semiovale, as reported to be greater than 0.3 in prior studies (2,3,5).

The precision of the estimates of the present study appears quite low, as indicated by the 95% confidence limits not small compared to the intercepts of the regression. Although editing may provide complete differentiation between NAAG and NAA, the reliability is limited by low SNR. B_1 inhomogeneity effects may be detrimental in editing, given that the effects of flip angle variations differ between coupled and uncoupled spin signals (13). The artifacts can be reduced using adiabatic pulses for volume localization (14). Some errors could be caused by the negative tails of *in vivo* NAA edited signals, which appear more or less greater than in phantom spectra. In this case, a slightly longer TE (e.g., 113 ms) may be preferable, at which negative tails may disappear, (compare Fig. 1). It is unlikely that frequency drifts during the editing scan, which were within ± 3 Hz, influenced NAAG editing yield under the Gaussian E180 of the present study substantially ($< 1\%$ as verified in phantoms). However, subject motions cause scanning an anatomy with different GM and WM contents, resulting in misleading NAAG signal intensity and inputs in subsequent data analysis.

For MEGA difference editing, signal reduction occurs extensively in the edit-off scan (subscans A' and B') due to chemical shift localization errors (15,16) when the slice-selection BW is insufficient. For the 180° pulses (BW = 1400 Hz) of the present study, whose BW was 2.2 times greater than the spectral distance between the NAAG coupling partners (*i.e.*, 4.61 – 2.5 ppm = 629 Hz at 7T), the chemical shift localization error can be ~50% relative to the slice thickness, leading to a virtually null signal in the edit-off scan. This slice displacement is much larger than in the prior 3T study (5), which used a 2.2 kHz BW 180° pulse for volume localization. The voxel displacement artifact also occurs in NAA editing, although signal reduction is slightly alleviated due to the smaller spectral distance between the coupling partners.

Several metabolites may be coedited in NAAG and NAA difference editing, as seen in Fig. 3. In NAAG editing, any metabolites having coupled resonances in the proximity of 4.61 and 4.15 ppm will be coedited. The 4.06 ppm resonance of myo-inositol is partially (50%) affected by the E180 when tuned at 4.15 ppm, giving a coedited signal of its coupling partner at 3.52 ppm. A negative peak at ~3.2 ppm may be attributable to phosphorylethanolamine as its coupling partner (3.98 ppm) is excited when E180 is tuned to 4.15 ppm. A small peak at ~2.95 ppm may be a coedited signal of the GSH cysteine CH₂ protons, which are coupled to the 4.56 ppm resonance. A signal at ~2 ppm is largely attributed to the NAAG glutamate moiety as its resonance at 4.13 ppm is excited by E180. The inconsistency of the 2-ppm multiplet pattern between the prefrontal and right frontal spectra is in part due to a cancellation error of the NAA CH₃ singlet. The Cr CH₂ resonance at 3.92 ppm is partially excited by E180 when tuned to 4.15 ppm, giving a signal at 3.92 ppm in difference spectra. In NAA editing, a peak at 3.65 ppm may be a coedited composite signal of glycerophosphorylcholine (GPC) and phosphorylcholine (PC). The 4.31 and 4.28 ppm resonances of the GPC choline moiety and PC are partially (70% and 47%) excited when the E180 is tuned to 4.38 ppm, resulting in coediting of their coupling partners at 3.66 and 3.64 ppm, respectively.

Acknowledgments

This research was supported by funds received from the State of Texas in support of the Metroplex Comprehensive Medical Imaging Center.

REFERENCES

1. Frahm J, Michaelis T, Merboldt KD, Hanicke W, Gyngell ML, Bruhn H. On the N-acetyl methyl resonance in localized ¹H NMR spectra of human brain in vivo. *NMR Biomed.* 1991; 4:201–204. [PubMed: 1657102]
2. Pouwels PJ, Frahm J. Differential distribution of NAA and NAAG in human brain as determined by quantitative localized proton MRS. *NMR Biomed.* 1997; 10:73–78. [PubMed: 9267864]
3. Vrenken H, Barkhof F, Uitdehaag BM, Castelijns JA, Polman CH, Pouwels PJ. MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. *Magn Reson Med.* 2005; 53:256–266. [PubMed: 15678547]
4. Tkac I, Andersen P, Adriany G, Merkle H, Ugurbil K, Gruetter R. In vivo ¹H NMR spectroscopy of the human brain at 7 T. *Magn Reson Med.* 2001; 46:451–456. [PubMed: 11550235]
5. Edden RA, Pomper MG, Barker PB. In vivo differentiation of N-acetyl aspartyl glutamate from N-acetyl aspartate at 3 Tesla. *Magn Reson Med.* 2007; 57:977–982. [PubMed: 17534922]
6. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed.* 2000; 13:129–153. [PubMed: 10861994]
7. Choi C, Dimitrov I, Douglas D, Zhao C, Hawes H, Ghose S, Tamminga CA. In vivo detection of serine in the human brain by proton magnetic resonance spectroscopy (¹H-MRS) at 7 Tesla. *Magn Reson Med.* 2009; 62:1042–1046. [PubMed: 19526507]

8. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed.* 1998; 11:266–272. [PubMed: 9802468]
9. Henry PG, Dautry C, Hantraye P, Bloch G. Brain GABA editing without macromolecule contamination. *Magn Reson Med.* 2001; 45:517–520. [PubMed: 11241712]
10. Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med.* 1993; 29:804–811. [PubMed: 8350724]
11. Ogg RJ, Kingsley PB, Taylor JS. WET, a T_1 - and B_1 -insensitive water-suppression method for in vivo localized 1H NMR spectroscopy. *J Magn Reson B.* 1994; 104:1–10. [PubMed: 8025810]
12. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, Merkle H, Ugurbil K, Chen W. Proton T_2 relaxation study of water, N-acetylaspartate, and creatine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T. *Magn Reson Med.* 2002; 47:629–633. [PubMed: 11948722]
13. Snyder J, Thompson RB, Wild JM, Wilman AH. Strongly coupled versus uncoupled spin response to radio frequency interference effects: application to glutamate and glutamine in spectroscopic imaging. *NMR Biomed.* 2008; 21:402–409. [PubMed: 17918776]
14. Klomp DW, Bitz AK, Heerschap A, Scheenen TW. Proton spectroscopic imaging of the human prostate at 7 T. *NMR Biomed.* 2009; 22:495–501. [PubMed: 19170072]
15. Slotboom J, Mehlkote AF, Bovee WMMJ. The effects of frequency-selective RF pulses on J-coupled spin-1/2 systems. *J Magn Reson A.* 1994; 108:38–50.
16. Yablonskiy DA, Neil JJ, Raichle ME, Ackerman JJ. Homonuclear J coupling effects in volume localized NMR spectroscopy: pitfalls and solutions. *Magn Reson Med.* 1998; 39:169–178. [PubMed: 9469698]

FIG. 1.

Phantom difference edited spectra of NAAG and NAA aspartate CH₂ resonances at 7T are plotted vs. TE. Spectra are broadened to singlet FWHM of 9 Hz. TE₁ and TE₂ were both 26 ms. Spectra are scaled with respect to the corresponding singlet amplitude.

FIG. 2.

Sub- and difference-edited spectra of NAAG and NAA difference editing, obtained from two phantoms. Subecho times were $(TE_1, TE_2, TE_3) = (26, 26, 56)$ ms. Spectra, broadened to singlet FWHM of 9 Hz, are normalized with the NAAG or NAA singlet. Vertical lines indicate the width for peak area calculation. TR was 10 s ($> 5T_1$).

FIG. 3.

Representative *in vivo* sub- and difference-spectra of NAAG and NAA editing, obtained from the medial prefrontal and the right frontal cortices are shown together with the voxel positioning (voxel size $25 \times 30 \times 30 \text{ mm}^3$). The spectra were apodized with a 2-Hz exponential function before Fourier transformation. Vertical lines indicate the width for peak area calculation. TR = 2.5 s. TE = 108 ms. NEX = 256 and 64 for NAAG and NAA editing, respectively.

FIG. 4.

Linear regression for the NAAG-to-NAA concentration ratios measured from the prefrontal and right frontal regions in five healthy volunteers vs. fractional white matter content. The dotted lines indicate 95% confidence intervals of the fit.