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Automated Whole-Brain N-Acetylaspartate Proton MR Spectroscopic Quantification

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Abstract

The neuronal-marker, NAA, a quantitative metric for neurons' health and density, is currently obtained by integration of the manually defined peak in whole-head ¹H-MRS. Our goal was to develop a full spectral modeling approach for estimating automatically the whole-brain NAA concentration (WBNA) and to compare the performance of this approach with a manual frequency-range peak integration approach previously employed. MRI and WBNA ¹H-MRS from 18 healthy young were examined. Their non-localized, whole-head ¹H-MRS obtained at 3 T yielded the NAA peak area through both manually-defined frequency-range integration and the new, full spectral simulation. The NAA peak area was converted into an absolute amount with phantom replacement and normalized for brain volume (segmented from *T*₁-weighted MRI) to yield the WBNA. A paired sample *t* test was used to compare the WBNA paradigms and a likelihood ratio test to compare their coefficients of variation. While the between-subject WBNA means were nearly identical: 12.8±2.5 mM for integration, 12.8±1.4 mM for spectral modeling, the latter's standard deviation was significantly smaller (~50%, *p*=0.026). The within-subject variability was 11.7% (1.3 mM) for integration, versus 7.0% (±0.8 mM) for spectral modeling, *i.e.*, a 40% improvement. The (quantifiable) quality of the modeling approach was high, as reflected by Cramer-Rao lower bounds <0.1% and vanishingly small (experiment - fit) residuals. Modeling the whole-head ¹H-MRS increases WBNA quantification reliability by reducing its variability, its susceptibility to operator bias and baseline roll, and by providing quality-control feedback. Together, these enhance the usefulness of the technique to monitor neurological disorders' diffuse progression and treatment response.

Keywords

MR spectroscopic quantification; MRI-segmentation; N-acetylaspartate (NAA); whole-brain NAA concentration (WBNA)

Introduction

Despite conventional MRI's exquisite sensitivity to soft-tissue morphology, its specificity to microscopic and especially metabolic pathology in otherwise “normal-appearing” brain tissue is limited (1). Consequently, ^1H -MRS is often added for specific biochemical information via the levels of several detectable neurometabolites and mobile lipids (2-4). Of these, the most diagnostic value is obtained from the amino-acid derivative, NAA (5, 6), which has putative roles in maintaining neuronal integrity, *i.e.*, through myelin production, protein synthesis, and as a storage for aspartate, as a precursor for *N*-acetyl-aspartyl-glutamate (NAAG) and as an osmolyte regulating intra-axonal water fraction of myelinated axons (6). First described in 1956 by Tallan *et al.* (7), NAA is almost exclusive to neurons and their processes (8-10) (with a <10% contribution coming from glia and the extracellular fluid (8, 11)), and consequently, considered a marker for their health and density (12). With the exception of Canavan disease, NAA concentration declines have been reported in many neurological disorders (5), and therefore is a leading candidate for noninvasive, *in vivo* assessment of the brain's neuronal status (5).

Comprising nearly 0.1% of the mammalian brain's wet weight, ~ 5.7 $\mu\text{moles/g}$ tissue (7, 13), NAA is the second most abundant free amino acid in the brain. Its *N*-acetyl CH_3 singlet at 2.02 ppm is the most prominent peak in the healthy brain's ^1H -MR spectrum (6, 14). However, with the exception of a few studies using “whole-brain” methods, [see recent review by Posse *et al.* (15)], almost all of the numerous ^1H -MRS studies to date used either small, 3 – 8 cm^3 , single-voxels or slightly larger two-dimensional chemical shift imaging-based VOIs (2, 16). These must be placed away from the skull to avoid the risk of contamination from subcutaneous lipid and bone marrow signals, thereby missing most of the cortex (17). In addition, due to their small size relative to the >1 liter brain, such single-voxel or chemical shift imaging VOIs must be image-guided onto MRI-visible pathologies (18), subjecting ^1H -MRS to the assumption that metabolic abnormalities occur only there. Since the most common neurological disorders, *e.g.*, multiple sclerosis, HIV infection, Alzheimer's disease and mild brain trauma, are diffuse (19), affecting all or most of the brain even during their sub-clinical periods (20, 21), techniques that miss 90 – 99% of the brain render estimates of the disorders' true extent prone to extrapolation errors (22).

Localized ^1H -MRS is also susceptible to VOI repositioning errors in serial studies, requires long acquisition times (few minutes for 3 – 8 cm^3 single-voxel studies and 20 – 30 min. for 1 cm^3 voxels) to yield sufficient SNR; and is susceptible to T_1 and T_2 weighting that are both rarely known in all brain regions and diseases. These issues are addressed by a non-localizing ^1H -MRS sequence that obtains the signal from the entire head to yield the whole-brain NAA (WBNA) concentration (23). The method has been shown to detect and follow global neuronal dysfunction in multiple sclerosis, HIV infection, mild traumatic brain injury, primary brain neoplasms, radiation therapy, Alzheimer's disease and normal aging (24-33). Its lack of explicit localization removes issues of VOI guidance, serial misregistration and long acquisitions since the whole-brain SNR is high (34). However, quantification with this sequence is not straightforward and has hereto been done by manual phasing, NAA peak-region definition, followed by peak-area integration (32), making it susceptible to operator and baseline bias (35).

In this paper we introduce a new approach to remove these biases and need for expert post-processing, using a novel, automated method that fits the whole-head ^1H spectrum with the full NAA (and additional ^1H -MRS-visible “nuisance” metabolites) spectral model functions, together with a baseline estimate. It estimates the NAA peak area and also yields, as data-quality metrics, its Cramer-Rao lower bounds, linewidth and (experimental – fit) residual. We compare the performance of the proposed WBNA quantification method with that of the previous manually-defined NAA peak integration in a cohort of young healthy adults.

Experimental

Human subjects

Eighteen healthy adults: 11 men, 7 women, 25 to 41 (33.2 ± 5.2) years-old were enrolled. Their demographics are compiled in Table 1. Each subject's “healthy” status was based on negative answers to 20 excluding neurological conditions before the scan and unremarkable MRI, confirmed by a board-certified neuroradiologist, afterward. All participants gave Institutional Review Board-approved written informed consent.

MRI and brain parenchyma volume - V_B

All experiments were done in a 3 T MR scanner (Trio, Siemens AG, Erlangen, Germany) using a circularly-polarized transmit-receive head-coil (MRInstruments Inc. Minneapolis MN). After placing each subject head-first supine into the scanner, sagittal T_1 -weighted 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) MRI were acquired for brain segmentation: TE/TR/inversion time (TI): 3.5/2150/1000 ms, 7° flip angle, 144 slices 1.1 mm thick, 256×224 matrix, and $256 \times 256 \text{ mm}^2$ field-of-view. Since the metabolites' concentrations in the cerebrospinal fluid are below the ^1H -MRS detection threshold (36), the subjects' V_B was obtained from their MP-RAGE images using our FireVoxel package (37), as shown in Fig. 1. Its precision for T_1 -weighted MRI has already been established at 3.4% (37).

Whole-head ^1H -MRSI

Following the MRI, we optimized the magnetic field ($\mathbf{B}_0$) homogeneity over the whole head by adjusting the scanner's first and second order shim currents, using a proton chemical shift imaging-based automatic procedure (38). The total amount of brain NAA, Q_{NAA} , was then obtained with a non-localizing TE/TI/TR=5.3/940/10⁴ ms ^1H -MRS sequence, shown in Fig. 2a (23, 39). This relies on both the lipids' $T_1 \approx 220$ ms (16) and macromolecules that resonate around 2.0 ppm whose $T_1 \approx 300$ ms (40, 41), both much shorter than the NAA's $T_1 \approx 1.35$ s (42, 43), to null just the latter with TI= $\ln 2 \times T_1 = 940$ ms inversion-recovery on even acquisitions, as shown in Fig. 2a, with an adiabatic inversion pulse designed for $\pm 50\%$ $\mathbf{B}_1^+$ inhomogeneity immunity, as shown in Fig. 2b (23). This strategy is chosen since it is only practical to null a single T_1 species (NAA) rather than the lipids and macromolecules that span a broad, 200 – 300 ms T_1 range, as shown by Hovener *et al.* (39). The cost of this strategy: loss of the NAA signal every second acquisition, is acceptable, since its whole-brain signal is large enough. Note that T_1 variations of up to $\pm 10\%$ of the nominal 1.35 s for which the TI nulling was designed, will affect the NAA M_z magnetization by less than 4%, as shown in Fig. 2c. Also note that spinal cord contributions to the WBNA signal are

negligible since its volume is small, a few cm³, or <1% of the brain's, as shown in Fig. 1; and coil sensitivity drop-off further suppresses that already small contribution.

Since the NAA-nulling TI=940 ms is much longer than T_1 s of lipids and macromolecules, their magnetization is at or near thermal equilibrium every acquisition, whereas NAA is fully relaxed only in odd (no inversion) but null at even (inverted) ones. Six water suppression pulses during the TI are followed by a binominal 1331 90° read-out that also provide more water and lipid rejection, as shown in Fig. 2d (44). Subtracting every even from odd transients cancels the short T_1 species (lipids and macromolecules), but not long- T_1 metabolites, as shown in Fig. 1 (23, 39). The $TR \gg T_1$ and $TE \approx 5$ ms make the sequence insensitive to T_1 and T_2 variations, which is desirable in pathologies where neither is likely to be known. At 8 add-subtract pairs averaged, the WBNA sequence adds <3 minutes to the protocol at excellent SNR, as shown in Fig. 1.

Quantification by manual NAA spectral peak integration

The NAA peak area, S_S , was integrated using in-house software written in IDL (Research Systems Inc. Boulder CO) after a trained operator's manual first and second order phasing and manual definition of its peak's left and right edges, p_1 and p_2 , as shown in Fig. 1. This procedure was repeated for each subject by four, blinded operators. If any of these four measurements of S_S was more than two standard deviations (SD)s from the four measurements' mean, it was rejected. If more than one S_S was rejected that data set was excluded. The 4 (or 3) "good" S_S were averaged into $\bar{S}_S$ and converted into absolute amounts, Q_{NAA} , in reference to a 2 L sphere of 1.5×10^{-2} mole NAA in water using subject and reference NAA peak areas, S_S and S_R (23),

$$Q_{NAA} = 1.5 \times 10^{-2} \cdot \frac{\bar{S}_S}{S_R} \cdot \frac{V_S^{180^\circ}}{V_R^{180^\circ}} \text{ moles, [1]}$$

with $V_R^{180^\circ}$ and $V_S^{180^\circ}$ the transmitter voltages for a non-selective 1 ms 180° inversion pulses on the sphere and subject, reflecting their relative coil loadings. To account for normal human brain size variation (45), each Q_{NAA} is divided by that individual's V_B to yield a global NAA concentration:

$$WBNA = Q_{NAA} / V_B \text{ mM, [2]}$$

a specific metric, independent of brain size and therefore, suitable for *inter*-subject comparison. Note that although several peaks are distinct in the whole-head ¹H-MRS of Fig. 1, only NAA is implicitly localized by its biochemistry to neurons only, *i.e.*, to just the brain (6, 46). All others, most notably: glutamine (Gln), glutamate (Glu), total creatine (tCr), choline (tCho) and *myo*-inositol (mIns), are found in all tissue types, making it impossible to ascertain the brain's fraction of their signals. Furthermore, although other *N*-acetyl species also resonate around 2.02 ppm, most notably NAAG, these are accounted for in the NAA basis function, as described in the Automated Data Processing and Spectral Fitting for NAA quantification, below.

Finally, since the coil's transmit radio-frequency (RF) field, $\mathbf{B}_1^+$, affects the performance of the WBNA sequence's 1331 90° read-out pulse, it was mapped over the whole brain of one subject, using the method of Breton *et al.* (47). Briefly, a series of 8 fast gradient-echo images was acquired for each slice with a sequence comprising a non-selective, short, 500 μ s, rectangular (to minimize sensitivity to $\mathbf{B}_0$ inhomogeneity) preparation pulse of nominal flip angle, θ_{nom} , ranging from $0^\circ - 140^\circ$ in 20° increments, followed immediately by a spoiler gradient and single-shot readout. All images had $2 \times 2 \text{ mm}^2$ in-plane resolution, 10 mm thick slices, 490 Hz/pixel receiver bandwidth, 1.23 ms TE, 3.0 ms echo spacing, low, 5° , readout flip angle, centric order phase encoding to minimize T_1 weighting and 10 s TR to allow full magnetization recovery. Twelve slices were needed to cover the brain, as shown in Fig. 3a. $\mathbf{B}_1^+$ maps were obtained for each slice by fitting every voxel's signal intensities, S , as a function of θ_{nom} :

$$S = S_0 \cos(\beta \cdot \theta_{nom}), \quad [3]$$

With S_0 , the proton-density weighted signal and β the actual/nominal $\mathbf{B}_1^+$ the fitted parameters. Variations in β thus represent a measure of $\mathbf{B}_1^+$ inhomogeneity, as shown in Fig. 3a (47).

Spectral Simulation of Metabolite Basis Sets

^1H -MRS spectral simulation and automated post-processing were done in VeSPA, an open source, downloadable software package (48, 49). A full density matrix-based spectral simulation was performed for each metabolite in the VeSPA-Simulation application using the sequence's actual complex RF pulse waveforms and timings, to account for linear and non-linear spectral phase distributions accumulated due to the 5 ms pre-acquisition delay and 1331 RF pulse.

Automated Data Processing and Spectral Fitting for NAA quantification

Automated data processing and spectral fitting were done in VeSPA-Analysis, a GUI based interactive program. The ^1H -MRS data were extracted in the instrument format and a standard set of preset processing and spectral fitting parameters applied as described below. Even FIDs were subtracted from the odd and the results summed into a single FID, read into VeSPA-Analysis and processed into spectra as follows: (i) an HLSVD-Pro algorithm was used to remove residual water signals at and above 4.0 ppm (50); (ii) a 1 Hz Gaussian apodization was applied; (iii) spectra were fitted using the automated algorithm described previously (51, 52).

For robust estimate of the NAA peak area, S_s , of Eq. [1], the NAA basis function included a signal contribution for NAAG, which resonates 0.04 ppm (~ 5 Hz) downfield and cannot, therefore, be resolved in this experiment, as shown in Fig. 4. This was added at a 1:0.15 ratio (15%) based on the work of Pouwels *et al.* (53). A combined model was chosen over independent functions due to the close proximity of the NAA and NAAG singlet peaks rendering the result obtained (by both methods) representative of the total-NAA. The metabolite basis set for the parametric fit also included basis spectra calculated for Glu, Gln, tCho, tCr and mIns. These were treated as “nuisance signals” and simplified the use of

wavelet filtering to account for non-parametric residual baseline signals. Examples of all basis functions are shown in Fig. 4 with 3 Hz Gaussian broadening and 1 Hz/point spectral resolution. Note that each plot represents the spectral pattern for one molecule of a metabolite rather than any specific concentration.

The absorption phased NAA peak at 2.01 *ppm* was shifted to match a simulated spectrum with a peak at 2.01 *ppm* by maximizing the correlation of the real to simulated data across a ± 0.1 *ppm* range. A B_0 shift term was then allowed to vary by ± 8 Hz in the optimization. All spectral fits began with an initial 8 Hz linewidth, determined empirically, with a 1-28 Hz optimization variability for a Voigt lineshape. Zero order phase shift was set to a fixed value for all data sets, determined empirically from basis set simulation, and allowed to vary $\pm 45^\circ$ in the optimization. First order phase shift was set to a negligible 0.1° and was allowed to vary by $\pm 2000^\circ$. The starting value for NAA area was estimated from the NAA peak height and line width estimates measured from the raw data (54). “Nuisance metabolite” areas were set as a ratio to the NAA starting value based on empirically determined scaling values applied equally across all data sets. The wavelet filter used to characterize baseline signal contributions was set to reject all signals under twice the metabolite linewidth for the current iteration. Ten iterations were done between the parameterized metabolite model and the non-parametric wavelet baseline steps.

Statistical analyses

A likelihood ratio test for the comparison of variance components in a mixed model was used to compare the two sets of WBNA measures in terms of their coefficient-of-variation ($CV = SD/mean$). Each set of measures was normalized by dividing each observation by its mean. As a result, the likelihood ratio test to compare the variances of the normalized measures corresponds to a test of whether the two sets have the same CV. The dependent variable was the vector of both sets of normalized WBNA measures. The model included data set [“WBNA1” (integration) versus “WBNA2” (fit)] as a fixed classification factor and subject ID was incorporated into the analysis as a random classification factor to account for the within-subject correlation due to the measures being made on the same subjects. Statistical tests were conducted at the two-sided 5% significance level using SAS 9.3 (SAS Institute, Cary, NC).

Results

Our automatic shimming procedure yielded a consistent 27 ± 4 Hz full width at half height whole-head water linewidth in under 5 minutes in these 18 subjects. Although this linewidth is reasonable for such a large, >1 liter, heterogeneous tissue volume at 3 T, there are also regions in the inferior frontal lobes, lateral and inferior temporal lobes that are known to experience more substantial broadening and shifts due to susceptibility differences. It has been shown, however, that these lead only to a $11 \pm 1\%$ *under*-estimation of the total brain NAA amount (55).

The map of the coil's B_1^+ variations over the whole brain is shown in Fig. 3a and a histogram of its values, in the 32,235 pixels in all 12 slices, is shown in Fig. 3b. It reveals a mean B_1^+/B_1^+ (nominal) ratio of 0.973 ± 0.093 , *i.e.*, $\pm 10\%$, but since the distribution of Fig.

3b is not normal, we also assessed its median: 0.99, with a 0.93 and 1.04 for the lower and upper quartiles. Less than 18% of all pixels experienced $B_1^+ < 90\%$ of nominal. Since, (i) the coil's head-cup ensures that all heads are placed in the same position inside; (ii) human brain volumes are similar to within $\pm 10\%$ (45) and (iii) the dielectric properties of the human head, to within the sensitivity of the MRS experiment, are the same for all healthy individuals, we assume that this B_1^+ distribution pertains to all subjects. To gauge its effects on the 133 L pulse that “reads-out” the longitudinal magnetization we plotted the transverse, M_{XY} , magnetization, obtained by numerical simulations of the Bloch equations in the presence of relaxation ($T_1=1350\text{ ms}$, $T_2=300\text{ ms}$) for 0% , $\pm 10\%$ B_1^+ variations, in Fig. 3c. It shows that in the worst case scenario: $B_1^+ = 90\%$ of nominal and at the “edge” of the NAA chemical shift, its signal suffers *only* an 8% loss. It is noteworthy that, as stated above, only $\sim 10\%$ of the brain volume will “see” B_1^+ variations that large (*cf.* Figs. 3a and 3b), making their overall effect relatively negligible, and importantly, consistent for all subjects.

The V_B -s needed to normalize the subjects' Q_{NAA} , are compiled in Table 1. The WBNAAs values obtained from Eqs. [1] and [2] using peak-integration and full spectral fitting are also given in Table 1, with the NAA linewidths, a by-product of the fitting process; and their distributions, shown in Fig. 5. An additional output is an estimate of the Cramer-Rao lower bounds for each metabolite (56), that due to the extremely high SNR, were all consistently reported to be $< 0.1\%$ of the NAA and nuisance metabolites' areas. After adjusting the individual WBNAAs values of the automated fitting method with the fixed 15% NAAG signal contributions added to the NAA basis function, the estimated WBNAAs means of the two post-processing methods were not significantly different (12.83 versus 12.80 mM; $p=0.691$). The 2.5 mM between-subject SD for the manual integration approach ($CV=20\%$) was significantly higher than the 1.4 mM ($CV=11\%$) for the full spectral modeling ($p=0.026$), as shown in Table 1 and Fig. 5a. To underscore this approximately twofold improvement, we also plotted the distribution of each individual's WBNAAs – the group's mean WBNAAs, as shown in Fig. 5b.

Spectral fitting results (experiment, fit and baseline) are shown in Fig. 6a. They were chosen to demonstrate the three most common whole-head ^1H -MRS scenarios: (i) significant residual lipids' signal; (ii) significant residual water signal; and (iii) minimal lipid and water signals. The (experiment – fit) residuals, in Fig. 6b, demonstrate the quality of the fit. Note that the spectral integration approach used previously does not provide such quality-control feedback. The VeSPA simulated basis functions used in the fit are shown in Fig. 6c. The fitted linewidths, another data quality metric, range from 8.4 to 12.5 Hz with fairly similar performance.

To assess the proposed quantification method's within-subject reproducibility, we scanned three healthy young males (to minimize possible gender and age variations: numbers 16 – 18 in Table 1), one after the other and repeated this three times, on the same day, to minimize possible temporal *within- and between-*subject biological variations. The results from one subject's three scans, post-processed with the proposed spectral modeling, are shown in Fig. 7. Their metrics reveal an average *within*-subject WBNAAs CV of 11.7% for manual peak integration [similar to 8 – 12% reported previously using several expert readers (23, 32, 39, 57)], versus 7% for spectral modeling, *i.e.*, over 40% improvement. As expected, these 7%

within-subject variations are much smaller than the 11% *between*-subject variability using the same fitting method.

Discussion

Although the WBNA method was initially introduced in the late '90s (23) – and has since been used in studies of several diffuse neurological disorders (34) – its acceptance, however, has been limited to just few laboratories worldwide (27, 31, 58). This may be due, at least in part, to the need for: (i) the custom ^1H -MRS WBNA sequence of Fig. 2; (ii) MRI segmentation software, *e.g.*, that used in Fig. 1; but especially for, (iii) custom post-processing software and, hereto, (iv) expert personnel to zero- and first-order phase the spectra and to identify the peak edges over an undulating baseline (35). The prevalence and ubiquity of free, downloadable MRI brain segmentation packages over the past decade (59, 60) have addressed issue (ii) above, *i.e.*, obtaining reproducible estimates of V_B for Eq. [2]. The goal of this study, therefore, is to devise and demonstrate a robust, *automated*, spectral fitting method for WBNA quantification that is operator and baseline-bias-free, to address issues (iii) – (v), above.

The central obstacles for metabolic (NAA) spectral-quantification in this non-localizing ^1H -MRS sequence are that the metabolites' signal contributions are complicated by: (i) spectrally varying phase; (ii) baseline that contains varying contributions from residual water and lipids' signals; (iii) “nuisance” metabolite signals; and (iv) semi-random global $\mathbf{B}_0$ distributions and time-varying shifts from subjects' breathing or motion that may lead to additional spectral distortions. In this report, we focused only on robust estimation of the WBNA signal area in the presence of complexities (i) – (iv) above.

To determine whether the new approach realized this goal we compared its performance with the previous simple integration of the manually-phased-and-edges-defined NAA peak (32, 57). The two methods yield nearly identical mean WBNA ($\sim 1\%$ apart), with SDs that although smaller for the spectral modeling approach, are nevertheless not statistically different either. Since the reproducibility of WBNA peak integration approach is already well established (23, 32, 39, 57, 61), these similarities validate the proposed spectral-fitting paradigm. The automation, however, in addition: (i) removes the need for several expert operators and also yields several quality control metrics that are not available with the simple integration approach, namely: (ii) Cramer Rao bounds for each metabolite (56); (iii) (experiment-fit) residuals; and (iv) metabolites' linewidths. These advantages are demonstrated in Fig. 5, where an outlier using the integration technique was nevertheless captured correctly by the spectral fitting approach.

The use of Glu, Gln, tCr, tCho and mIns in the basis-set used to parameterize known signal contributes did serve to simplify the non-parametric baseline signal estimated by the wavelet filtering method. Gln signal areas, however, were consistently reported close to zero, as shown in Fig. 6c. Therefore, Gln should possibly be excluded, or alternatively included as part of a Glu + Gln= “Glx” basis function, often reported as a 5:1 Glu:Gln (62). The high SNR of the “nuisance signals” dominates the interactions between the metabolite and baseline iterations of the optimization. Therefore, some form of quality measure, *e.g.*,

confidence intervals (63), that include a description of the metabolite model, as well as the data itself as part of their calculation, might prove more useful.

This fitting approach may be improved in several possible ways. First, it may prove beneficial to exclude individual FID pairs that were corrupted by subjects' breathing or motion. Second, due to the high SNR, it may be feasible to individually analyze the WBNA data from each FID pair. The average (or regression) of all these pairs could further minimize cross-contamination of the spectrum to be fitted by the summing of semi-random water and lipid signals and global B_0 shifts. Finally, it may even be possible to fit WBNA in each FID data pair simultaneously. This will enforce an average value for NAA while allowing variable baseline nuisance signals to be more flexibly estimated by the wavelet filter.

Despite the novel improvement in post processing, the WBNA approach still suffers from several inherent limitations. First, due to its maximal “partial volume” effects, the method is insensitive to NAA signal changes smaller than the $\sim 15\%$ between-subject sensitivity threshold. Second, the lack of implicit localization precludes structure, or region-specific, analyses and implicitly assumes that NAA changes, even when detected, are “homogeneous,” *i.e.*, uniform throughout the brain. Note, however, that most of the prevalent neurological conditions, *e.g.*, trauma, multiple sclerosis, HIV infection, cancer and Alzheimer's disease fall into this global diffuse category that affects the entire brain (34), although WBNA spectra from such pathologies are beyond the scope of this study and, therefore, are not shown here.

In conclusion, full spectral computer modeling of whole-head ^1H -MRS yields *inter*-subject WBNA distribution similar to that obtained using manually-defined spectral region integration but without a need for (several) expert operators and yields quantitative reliability metrics: Cramer-Rao lower bounds, (experiment – fit) residual and linewidth. In addition, the approach reduces the within- and between-subject variances, increasing the statistical power of the methodology. These new advantages, in addition to the speed (less than 5 minutes) of acquisition, may enhance the usefulness of the WBNA approach to monitor the global progression and treatment effects of diffuse neurological disorders.

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Abbreviations

^1H-MRS	Proton MR spectroscopy
BW	bandwidth
FID	free induction decay
NAA	<i>N</i> -acetyl-aspartate
NAAG	<i>N</i> -acetyl-aspartate-glutamate
RF	radiofrequency
SNR	Signal-to-noise ratio
VeSPA	Versatile Simulation, Pulses and Analysis
VOI	Volume of interest
WBNA	whole-brain NAA concentration

Fig. 1.

Left: Sample T_1 -weighted MP-RAGE images from subjects 1, 5 and 13 from Table 1, overlaid with their brain parenchyma mask (red) obtained with the FireVoxel MRI-segmentation package and used to estimate the brain parenchyma volume, V_B , for Eq. [2]. Note the masks' excellent capture of the tissue (avoiding cerebrospinal fluid, dura, fat and skull) and the relatively little atrophy that is characteristic of healthy young adults. Right: Whole-head ^1H -MRS spectra from these subjects demonstrating three scenarios: significant residual lipids' signal (top), significant residual water signal (center) and minimal lipid and water signals (bottom), all on the same intensity and chemical shift scales. Note that of all the visible metabolites peaks, most notably Glu, tCr and tCho, only NAA's is implicitly localized by its biochemistry to just the brain. Subject's NAA peak area, S_5 (cross-hatched), for Eq. [1], was obtained by manual definition of the peak's left and right edges, p_1 and p_2 , by the operator followed by numerical integration.

Fig. 2.

Top a: Schematic representation of the NAA nulling sequence comprising: (i) A 10 ms hyperbolic-secant RF adiabatic inversion [1 kHz peak B_1 , 50% adiabaticity, 1 kHz bandwidth (± 200 Hz at 98% inversion, shown in **b** below) centered -2.7 ppm upfield from the water] each even acquisition (64) (ii) TI=940 ms to null the NAA signal; (iii) numerically optimized six Gaussian RF pulses 44.5, 41.3, 47.3, 37.5 and 16 ms apart for 70 Hz BW water suppression (WS - flip angles denoted above each pulse); and (iv) a binominal 1331 (120 μ s rectangular pulses, 1.9 ms delays, with excitation profile shown in **c**) for a 90° readout (44, 65); and finally (v) after a 5 ms delay to suppress broad lipids components, 1 second acquisition for 1 Hz spectral resolution at ± 1 KHz BW. The sequence is repeated 16 times (8 add-subtract pairs) for 2 minutes, 40 seconds. **Bottom b:** M_z magnetization (dashed line) following the adiabatic inversion recovery. **c:** M_z magnetization (solid black line) following an inversion recovery, $M_z(t) = M_{z\infty}[1 - 2\exp(-t/T_1)]$ for a metabolite with a T_1 of 1350 ms, e.g., the NAA (42, 43). Note that a $\pm 10\%$ deviation in T_1 (dashed arrows) due to pathology or normal biological variations, will lead to less than 3.5% variation of M_z from a null (gray zone). **d:** Transverse M_{xy} for the binominal 1331, obtained by numerical simulations of the Bloch equations in the presence of relaxation. Hatched column indicates the 2 – 3.6 ppm range of the NAA - mIns chemical shifts. Note the near ideal inversion,

immune to $\pm 50\%$ $\mathbf{B}_1^+$ inhomogeneity of the adiabatic inversion and the coverage of the 2.0 – 3.7 ppm chemical shift range by the 1331 (see $\mathbf{B}_1^+$ inhomogeneity effects on it in Fig. 3c).

Fig. 3.

Left, a: Plot of twelve 1 cm thick proton density images from the vertex (top, left) down to the foramen magnum (bottom, right), of a volunteer, superimposed with their B_1^+ / B_1^+ (nominal) map, obtained as described in the Methods. Note the 0.973 ± 0.093 distribution of this metric over most of the cerebrum with only slight drop-off at the level of the cerebellum. Right, b: Histogram of the B_1^+ / B_1^+ (nominal) values in all the 32,235 pixels in **a** at 2.5% per bin. Note that $\sim 83\%$ of pixels “see” a B_1^+ / B_1^+ (nominal) > 0.90 . Right, c: The effect of B_1^+ variations on the transverse M_{XY} magnetization (WBNA resonance chemical shift range denoted by vertical gray zone) read by the 1331 pulse given 0%, $\pm 10\%$ variations, obtained by numerical simulations of the Bloch equations in the presence of relaxation ($T_1=1350$ ms, $T_2=300$ ms). Note that the worst case scenario: $B_1^+=90\%$ of nominal, at the “edge” of the NAA chemical shift ($\pm$ one linewidth), will suffer *only* 8% signal loss.

Fig. 4.

The tCho, tCr, Glu, Gln, mIns and NAA basis functions, synthesized using VeSPA-Simulation. Each basis function demonstrates the spectral pattern for one molecule of the given metabolite, except for the NAA which represents the NAA+NAAG at a 7:1 ratio (53), rather than any specific concentration. Note that the 1331 pulse and 5 ms pre-acquisition delay, shown in Fig. 2, lead to significant first-order phase twists and peak intensity variations in the spectra that are, however, accounted for in each metabolite's spectrum with spectral modeling of the complete sequence in Fig. 1. Note the NAAG partial overlap contribution (indicated with arrow) to the NAA peak, 5 Hz upfield, that is (still) visible with the 3 Hz line broadening used in the simulation.

Fig. 5.

Top, a: Box plots showing the 1st, 2nd (median), and 3rd quartiles (box), ± 95 percentiles (whiskers) and outlier (*) of the WBNA A distributions for the same subjects obtained for the manually-defined peak area integration and the full spectral fitting, overlaid with dot plots showing the $WBNA A_i$ of each of the $i=1..18$ individuals for each method (O), together with lines connecting the same individual's data. Note the significant ($p=0.026$) narrowing of the distribution of the full spectral modeling approach compared with that of manual integration (CV reduced from 20% to 11%, despite nearly identical means) and the correct capture of the outlier from the integration approach (arrow). **Bottom, b:** The distribution of the residuals for each method (each individual's $WBNA A_i$ - the group's mean, $\overline{WBNA A}$) demonstrating the $\sim$ twofold improvement in the variance of the proposed automated full spectral modeling approach.

Fig. 6.

Automated processing and spectral fitting results for three subjects from Table 1, demonstrating three performance scenarios: significant residual lipids signal (left), significant residual water signal (center) and minimal lipid and water signals (right), all on the same intensity and chemical shift scales. **Top, a:** Whole-head ^1H -spectrum (thin black line) overlaid with the fitted data + baseline (thick gray line) and baseline only (dashed line). **Center, b:** Residual signals [data - (metabolites + baseline)]. **Bottom, c:** Individual metabolite contributions model functions used by VeSPA to produce the fitted and baseline spectra. Note: (i) the quality of the fit reflected by the fitted model functions on **a**; and (ii) the consequent vanishing residuals in **b**, indicating excellent metabolite and baseline modeling of VeSPA to the whole-head ^1H -MRS.

Fig. 7.

Left to right: Automated processing and full VeSPA spectral fitting results from subjects #16, 17 and 18 in Table 1. Top to bottom: Each of their three repeated scans: Meas #1, #2 and #3, all on the same intensity and chemical shift scales. Note the within-subject reproducibility of the experimental methodology and full spectral fitting approach, reflected by: (i) the fitted model functions indicating excellent metabolite and baseline modeling of VeSPA to the whole-head ^1H -MRS; and (ii) vanishing residual (experimental – fitted) at the bottom of each frame.

Table 1

Age, gender, brain volumes, manual and fitted estimated areas for the whole-brain NAA peak, and VeSPA-estimated Voigt linewidth (LW) for all subjects (#s 16 – 18 were each scanned 3 separate times on the same day)

Subject	a Age/gender	V _B [cm ³]	b c WBNA (integration)	b d WBNA (spectral fit)	LW [Hz]
1	39/M	1390	15.9	15.3	9.5
2	34/F	1095	12.3	14.5	11.0
3	29/F	1161	20.2	13.4	11.5
4	29/F	1126	14.3	13.3	10.6
5	33/M	1357	14.7	13.4	12.7
6	30/F	1499	13.3	13.8	11.6
7	29/M	1537	11.5	10.5	10.5
8	25/F	1300	12.2	15.1	11.0
9	40/M	1372	10.8	12.7	10.5
10	39/M	1253	11.1	12.7	11.8
11	41/M	1374	9.7	11.6	9.2
12	29/F	1358	12.4	11.9	11.0
13	31/M	1485	10.1	10.2	10.0
14	41/M	1477	13.5	12.3	9.8
15	31/F	1229	10.9	10.9	13.4
16	35/M	1197	13.8	12.5	13.4
	"	"	10.4	12.6	13.7
	"	"	10.8	12.8	11.5
17	36/M	1205	16.1	13.1	11.8
	"	"	13.5	14.1	10.9
	"	"	16.6	14.7	11.5
18	26/M	1341	11.6	10.6	13.4
	"	"	11.7	11.8	11.8
	"	"	10.5	13.3	11.0
Mean:	33	1320	12.8	12.8	11.4
SD:	5.2	133	2.5	1.4	1.3

Subject	<i>a</i> Age/gender	<i>V_B</i> [cm ³]	<i>b</i> <i>c</i> WBNA (integration)	<i>b</i> <i>d</i> WBNA (spectral fit)	LW [Hz]
CV(%):	16%	10%	20%	11%	11%

^a M=male, F=female,

^b millimoles/g wet weight (NAA+NAAG) from Eq. [2],

^c peak integration,

^d ¹H-SPCA full spectral modeling. Mean, SD and CV (=SD/mean) for each column are shown at the bottom. Note the similar average (a basic requirement) and smaller variance (although statistically insignificant) for computer fitting compared with spectral integration.