Errors in $^1$H-MRS estimates of brain metabolite concentrations caused by failing to take into account tissue-specific signal relaxation

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Accurate measurement of brain metabolite concentrations with proton magnetic resonance spectroscopy ($^1$H-MRS) can be problematic because of large voxels with mixed tissue composition, requiring adjustment for differing relaxation rates in each tissue if absolute concentration estimates are desired. Adjusting for tissue-specific metabolite signal relaxation, however, also requires a knowledge of the relative concentrations of the metabolite in gray (GM) and white (WM) matter, which are not known a priori. Expressions for the estimation of the molality and molarity of brain metabolites with $^1$H-MRS are extended to account for tissue-specific relaxation of the metabolite signals and examined under different assumptions with simulated and real data. Although the modified equations have two unknowns, and hence are unsolvable explicitly, they are nonetheless useful for the estimation of the effect of tissue-specific metabolite relaxation rates on concentration estimates under a range of assumptions and experimental parameters using simulated and real data. In simulated data using reported GM and WM $T_1$ and $T_2$ times for N-acetylaspartate (NAA) at 3 T and a hypothetical GM/WM NAA ratio, errors of 6.5–7.8% in concentrations resulted when $TR = 1.5$ s and $TE = 0.144$ s, but were reduced to less than 0.5% when $TR = 6$ s and $TE = 0.006$ s. In real data obtained at $TR/TE = 1.5$ s/0.04 s, the difference in the results (4%) was similar to that obtained with simulated data when assuming tissue-specific relaxation times rather than GM–WM-averaged times. Using the expressions introduced in this article, these results can be extrapolated to any metabolite or set of assumptions regarding tissue-specific relaxation. Furthermore, although serving to bound the problem, this work underscores the challenge of correcting for relaxation effects, given that relaxation times are generally not known and impractical to measure in most studies. To minimize such effects, the data should be acquired with pulse sequence parameters that minimize the effect of signal relaxation.

KEYWORDS
brain, magnetic resonance spectroscopy, metabolite relaxation

Abbreviations used: CSF, cerebrospinal fluid; $d_x$, water density in compartment $X$; $f_{CSF}$, CSF water mole fraction; $f_{CSF,vol}$, CSF tissue volume fraction; $f_{GM}$, gray matter tissue water mole fraction; $f_{GM,vol}$, gray matter tissue volume fraction; $f_{WM}$, white matter tissue water mole fraction; $f_{WM,vol}$, white matter tissue volume fraction; GM, gray matter; $[H_2O]_{mol}$, molarity of pure water; $[H_2O]_{mol,vol}$, molarity of pure water; $#H_{na}$, number of metabolite hydrogen atoms; $[M]_{mol,vol}$, molarity of metabolite; $[M]_{mol}$, molarity of metabolite; MPRAGE, magnetization prepared rapid gradient echo; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; $[M]_X$, molarity of metabolite in tissue X (GM or WM); NAA, N-acetylaspartate; ROI, region of interest; $R_x$, relaxation rate in compartment $X$; $S_{H_2O}$, water signal intensity; $S_{M}$, metabolite signal intensity; WM, white matter.
Proton magnetic resonance spectroscopy (1H-MRS) in studies on the human brain requires relatively large voxels because of the low intensities of the metabolite signals. This poses a challenge for the estimation of metabolite concentrations in exclusively gray matter (GM) or white matter (WM) regions of interest (ROIs), as the voxels usually contain contributions from both GM and WM and, particularly in GM ROIs, cerebrospinal fluid (CSF). Several strategies exploiting spectroscopic imaging methods have been developed to estimate metabolite levels in pure GM or WM. However, these methods are not suitable for single-voxel studies using conventional volume selection. The application of the many variations of these sequences, including popular spectral editing techniques that often require especially large voxels, by far dominates the use of 1H-MRS in studies on the brain.

Although mixed tissue contributions present a potential confound for the interpretation of single-voxel 1H-MRS of the brain, the determination of the composition of the voxel by segmentation of a co-registered anatomical image allows correction for CSF, usually considered to be void of metabolites of interest at detectable levels, as well as the possibility of entering the tissue fraction of interest as a covariate in statistical analyses. Furthermore, if ‘absolute’ concentrations are to be estimated, a knowledge of the partial volume fractions allows the possibility to correct for compartment-specific effects of relaxation on the observed signals. This is particularly important when using the water signal as a concentration standard, as the water proton T1 and T2 relaxation times in GM, WM and CSF differ markedly. Owing to the greater technical challenges of measuring the relaxation rates of metabolite signals (weak, overlapping and often J-coupled), reports of their values have been inconsistent and, moreover, lacking for all metabolites of interest. However, when reported, the GM–WM differences have been small (reviewed by Di Costanzo et al.). Consequently, these differences have been ignored in most studies reporting concentration estimates of neurometabolites.

A formula for the estimation of molal concentrations (moles/kilogram of tissue water) of neurometabolites that incorporates the effects of compartment-specific relaxation on the water signal has been described. However, this formula does not account for tissue-specific effects of relaxation on metabolite signals but, instead, assumes that these rates are sufficiently similar in GM and WM to incorporate them as a single factor. Here, we extend the formula to include distinct metabolite relaxation terms and propose a similar equation for concentration estimates based on molarity (moles/liter of tissue). To our knowledge, an expression that correctly accounts for GM- and WM-specific metabolite relaxation in concentration estimates has not been reported previously. The modified equation necessarily contains two unknown variables and, as such, is not solvable explicitly. Nonetheless, it is useful for the examination of the impact of differing GM and WM metabolite relaxation rates on the estimate of metabolite concentrations over a range of MRS parameters and assumptions.

2 | THEORY

Molarity is concentration expressed in moles of solute per kilogram of solvent. In brain tissue, the solvent is water. The advantages of measuring molarity in brain 1H-MRS experiments have been discussed previously and include insensitivity to temperature, barometric pressure and the contribution of other solutes or non-soluble material to the solution. If the 1H-MRS signals are acquired under fully relaxed conditions (TE < T2, TR > T1), the molality of the metabolite protons is directly proportional to the ratio of the metabolite signal intensity SM to the water signal intensity S H2O, without assumptions about the water density in the tissue, as is required with molarity. In the simplest case of a 1H-MRS voxel sampling only pure GM or pure WM, molality is given by:

\[
[M]_{molar} = \frac{S_M}{S_{H2O}} \times \frac{2}{#H_M} \times [H2O]_{molar}
\]

where \([H2O]_{molar}\) is the molality of pure water (55.51 mol/kg water), \(#H_M\) is the number of metabolite hydrogen atoms, 2 is the number of water protons, and both the water and metabolite fractions are assumed to be fully detected. If CSF is present in the voxel, the water signal is scaled by the tissue water mole fraction \(f_{GM}\) or \(f_{WM}\) or, equivalently, \((1 - f_{CSF})\), where \(f_{CSF}\) is the CSF water mole fraction.

If the signals are not acquired under fully relaxed conditions, they must also be scaled by the appropriate T1- and T2-weighted relaxation factors. If the initial pulse angle is 90° in a double spin echo or stimulated echo type of experiment with equal echo spacing, this factor is \(R_x = \exp(-TE/T_{2x,y})[1 - \exp(-TR/T_{1x,y})]\), where the subscript \(x\) refers to either the metabolite or water signal and \(y\) refers to the compartment (GM, WM or CSF). Although different protons on a metabolite can have different relaxation times, here we assume that the times associated with the dominant peak approximately characterize the entire signal [e.g. the N-acetyl peak of N-acetylaspartate (NAA)]. This is an important caveat, but one that could be avoided only with (1) a knowledge of individual proton relaxation times which is, because of the difficulty in their measurement, lacking in the field; and (2) an accurate modeling of the total signal from each metabolite that takes into account any differing relaxation attenuation among its various spectral peaks. In the general case of partial volume contributions from GM, WM and CSF, each with a compartment-specific water proton relaxation factor \(R_{H2O,GM}, R_{H2O,WM}, R_{H2O,CSF}\), but assuming a common GM and WM metabolite relaxation factor \(R_{x0}\), the molality is given by.
\[ |M|_{molal} = \frac{S_{GM,obs} \times (f_{GM} \times R_{H2O,GM} + f_{WM} \times R_{H2O,WM} + f_{CSF} \times R_{H2O,CSF})}{S_{H2O,obs} \times (1-f_{CSF}) \times R_m} \times \frac{2}{\#H_m} \times [H_2O]_{molal} \tag{2} \]

The fractions in Equation 2 are not the volume fractions estimated by image segmentation, but the mole fractions of water. They can be related to the volume fractions by taking into account the relative water fraction in each segmentation fraction:

\[ f_{GM} = \frac{f_{GM,vol} \times d_{GM}}{f_{GM,vol} \times d_{GM} + f_{WM,vol} \times d_{WM} + f_{CSF,vol} \times d_{CSF}} \tag{3} \]

\[ f_{WM} = \frac{f_{WM,vol} \times d_{WM}}{f_{GM,vol} \times d_{GM} + f_{WM,vol} \times d_{WM} + f_{CSF,vol} \times d_{CSF}} \tag{4} \]

and

\[ f_{CSF} = \frac{f_{CSF,vol} \times d_{CSF}}{f_{GM,vol} \times d_{GM} + f_{WM,vol} \times d_{WM} + f_{CSF,vol} \times d_{CSF}} \tag{5} \]

where \( f_{GM,vol}, f_{WM,vol} \) and \( f_{CSF,vol} \) are the GM, WM and CSF volume fractions, respectively, estimated by segmentation, and the density factors (\( d \)) are the water densities associated with them.

Accounting for tissue-specific metabolite signal relaxation in the equation for molality is analogous to accounting for compartment-specific water signal relaxation. However, a critical difference is that, although the water molal concentration is the same in all compartments (i.e. the concentration of water in pure water, 55.51 mol/kg water), the metabolite concentrations are not. The metabolite mole fractions are not simply related to the volume fractions by taking into account the relative water fraction in each segmentation fraction:

\[ \text{GM} \quad [M]_{GM} = \frac{f_{GM} \times [M]_{GM}}{f_{GM} \times [M]_{GM} + f_{WM} \times [M]_{WM}} \tag{6} \]

and

\[ \text{WM} \quad [M]_{WM} = \frac{f_{WM} \times [M]_{WM}}{f_{GM} \times [M]_{GM} + f_{WM} \times [M]_{WM}} \tag{7} \]

where the fractions \( f_{GM}, f_{WM} \) and \( f_{CSF} \) are the mole fractions of water given by Equations 3-5. The observed metabolite signal is equal to the sum of the mole fractions of the fully relaxed signal \( S_{M,obs} \) with each fractional signal weighted by its compartment-specific relaxation factor:

\[ S_{M,obs} = f_{GM} \times S_{M,GM} \times R_{M,GM} + f_{WM} \times S_{M,WM} \times R_{M,WM} \tag{8} \]

Here ‘R’ has been added to the subscript \( S_M \) of Equation 1 to emphasize that it is the total signal intensity (from all compartments) that would be observed under fully relaxed acquisition conditions. Solving for \( S_{M,R} \), we obtain:

\[ S_{M,R} = \frac{S_{M,obs}}{f_{GM} \times R_{M,GM} + f_{WM} \times R_{M,WM}} \tag{9} \]

To account for the GM and WM metabolite signal fractions that are weighted by different relaxation factors, the term \( R_m \) in Equation 2 is substituted by the denominator of Equation 9. This term, which we label \( R_{M,GM,WM} \), can be expanded in terms of Equations 3, 4, 6 and 7. Further, the unknown GM and WM concentrations can be expressed as a single ratio to obtain:

\[ R_{M,GM,WM} = \frac{f_{GM,vol} \times d_{GM} \times ([M]_{GM} / [M]_{WM}) \times R_{M,GM} + f_{WM,vol} \times d_{WM} \times R_{M,WM}}{f_{GM,vol} \times d_{GM} \times ([M]_{GM} / [M]_{WM}) + f_{WM,vol} \times d_{WM}} \tag{10} \]

Inserting this term into Equation 2, we obtain:

\[ |M|_{molal} = \frac{S_{GM,obs} \times (f_{GM} \times R_{H2O,GM} + f_{WM} \times R_{H2O,WM} + f_{CSF} \times R_{H2O,CSF})}{S_{H2O,obs} \times (1-f_{CSF}) \times R_{M,GM,WM}} \tag{11} \]

It should be noted that, if the water and metabolite signals are acquired under fully relaxed conditions, Equation 11 reduces to Equation 1 with the water signal scaled by the tissue fraction, i.e. \( [M]_{molal} \) is independent of the metabolite concentrations in each tissue. As with the water mole fractions, the metabolite mole fractions in the calculation of molality serve only to account for how much of the total metabolite signal is scaled by each compartment-specific relaxation factor. Hence, at very short TE and sufficiently long TR (when \( R \approx 1 \)), the observed signal is simply...
proportional to the number of moles of metabolite and tissue water in the voxel. In addition, when the metabolite relaxation times are the same in GM and WM, Equation 11 reduces to Equation 2. These results are expected, of course: under simplifying conditions, Equation 11 must reduce to the simpler expressions. The validity of alternative strategies for the inclusion of differing GM–WM metabolite relaxation rates in partial volume corrections\(^\text{16,17}\) should also be evaluated with this requirement in mind.

An expression similar to Equation 2 can be written for molarity (moles per volume of tissue) by taking into account that the fractional contribution to \(S_{\text{H}_2\text{O}}\) from each compartment is weighted not only by the compartment-specific relaxation terms when calculating moles per volume of tissue, but also by the relative water densities in each compartment. This effectively scales the molarity of water in pure water \([\text{H}_2\text{O}]_{\text{molar}}\) (55.51 mol/L water) to the number of moles of water in the voxel volume. Again, starting with the simplest case of either pure GM or pure WM under fully relaxed conditions, molarity is given by:

\[
[M]_{\text{molar}} = \frac{S_{\text{H}_2\text{O}} \times d_{\text{tissue}} \times 2}{\# \text{H}_2\text{O}}
\]

(12)

In a voxel with a mixture of GM, WM and CSF not sampled under fully relaxed conditions, the observed water signal \(S_{\text{H}_2\text{O,obs}}\) is the sum of the volume fractions of the fully relaxed signal \(S_{\text{H}_2\text{O,R}}\) from each compartment, with each fractional signal weighted by the relaxation factor and the water density of the compartment:

\[
S_{\text{H}_2\text{O,obs}} = f_{\text{GM,vol}} \times d_{\text{GM}} \times S_{\text{H}_2\text{O,R,GM}} + f_{\text{WM,vol}} \times d_{\text{WM}} \times S_{\text{H}_2\text{O,R,WM}} + f_{\text{CSF,vol}} \times d_{\text{CSF}} \times S_{\text{H}_2\text{O,R,CSF}}
\]

(13)

As with the derivation of Equation 2, we solve for \(S_{\text{H}_2\text{O,R}}\) and substitute the resulting expression for \(S_{\text{H}_2\text{O}}\) in Equation 12. Assuming equal GM and WM metabolite signal relaxation rates at this point, substituting \(S_{\text{M,obs}}/R_M\) for \(S_M\) and scaling \(S_{\text{H}_2\text{O}}\) by the tissue volume fraction to correct for the CSF inclusion yields:

\[
[M]_{\text{molar}} = \frac{S_{\text{M,obs}} \times (f_{\text{GM,vol}} \times d_{\text{GM}} \times R_{\text{H}_2\text{O,GM}} + f_{\text{WM,vol}} \times d_{\text{WM}} \times R_{\text{H}_2\text{O,WM}} + f_{\text{CSF,vol}} \times d_{\text{CSF}} \times R_{\text{H}_2\text{O,CSF}})}{S_{\text{H}_2\text{O,obs}} \times (1-f_{\text{CSF,vol}} \times R_M)} \times \frac{2}{\# \text{H}_2\text{O}}
\]

(14)

where \([\text{H}_2\text{O}]_{\text{molar}}\) is the molar concentration of pure water (55.51 mol/L).

To account for different metabolite signal relaxation rates in GM and WM, the term \(R_M\) is replaced by \(R_{\text{M,GM,WM}}\) to yield:

\[
[M]_{\text{molar}} = \frac{S_{\text{M,obs}} \times (f_{\text{GM,vol}} \times d_{\text{GM}} \times R_{\text{H}_2\text{O,GM}} + f_{\text{WM,vol}} \times d_{\text{WM}} \times R_{\text{H}_2\text{O,WM}} + f_{\text{CSF,vol}} \times d_{\text{CSF}} \times R_{\text{H}_2\text{O,CSF}})}{S_{\text{H}_2\text{O,obs}} \times (1-f_{\text{CSF,vol}} \times R_{\text{M,GM,WM}})} \times \frac{2}{\# \text{H}_2\text{O}}
\]

(15)

3 METHODS

Equations 11 and 15 contain two unknown variables: the total tissue (GM + WM) metabolite concentration \([M]\) and the ratio of GM to WM metabolite concentrations (inherent in \(R_{\text{M,GM,WM}}\)). As such, they cannot be solved explicitly. However, approximations of the GM/WM metabolite ratio for various metabolites in healthy or diseased tissue can be obtained from many published reports. In most of these studies, spectroscopic imaging data were used to estimate concentrations in several voxels with mixed GM, WM and CSF content. Hypothetically pure GM and WM concentrations were estimated by regressing the concentrations against the fractional GM content of the voxel, normalized by the total tissue fraction, and extrapolating to fractions of 0 and 1 to estimate WM and GM concentrations, respectively.\(^\text{1}\) To demonstrate the impact of differing GM and WM metabolite \(T_1\) and \(T_2\) times on concentration estimates in the present article, we examine real and simulated spectroscopic imaging data, allowing us to display the effect over a range of tissue compositions typically encountered in brain \(^1\)H-MRS.

Simulated data were generated with programs written with Matlab (version R2014A, The MathWorks Inc., www.mathlab.com) by scaling hypothetical water and metabolite signals by the mole fractions and relaxation factors that would be associated with them in GM, WM and CSF. The GM and WM molar concentrations assigned to the metabolite signal fractions were 18 and 15 mol/kg tissue water, respectively, drawing from previous work,\(^\text{18}\) with each signal attenuated by relaxation factors based on \(T_1\) and \(T_2\) times reported previously for the N-acetyl protons of NAA in GM and WM\(^\text{19}\) (see the legend of Figure 1 for more details). To better mimic the situation of a slice transecting the inter-hemispheric fissure, an increasing fraction of CSF was included in the hypothetical voxels after the GM fraction exceeded the WM fraction, reaching a maximum of 15% when the WM was zero. In other words, voxels with no WM had 85% GM and 15% CSF, reflecting the common overlap of predominantly GM voxels with CSF spaces. Molar concentrations were plotted versus the normalized GM mole fraction \((f_{\text{GM}}/f_{\text{GM}} + f_{\text{WM}})\) and molar concentrations versus the normalized GM volume fraction \((f_{\text{GM,vol}}/f_{\text{GM,vol}} + f_{\text{WM,vol}})\).

NAA measurements were obtained from a healthy human subject in a previously reported study.\(^\text{18}\) Briefly, magnetic resonance imaging (MRI) anatomical and spectroscopic imaging data were acquired with a Siemens (Siemens Medical Solutions, 40 Liberty Boulevard Malvern, PA 19355 USA) 3-T Tim Trio scanner. \(^1\)H-MRS imaging was performed with a phase-encoded version of a double spin echo sequence, both with and without water presaturation [TE = 40 ms; TR = 1500 ms; slice thickness, 15 mm; field of view, 220 × 220 mm\(^2\); circular k-space sampling (radius = 12); total scan time, 582 s]. The MRS data were initially analyzed with LCMModel (http://s-provencher.com) and then with...
Matlab programs that applied either Equation 11 or 15. GM, WM and CSF segmentation maps were generated from a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) image using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). The study was conducted with the approval of the Institutional Review Board of the University of New Mexico Health Sciences Center.

4 | RESULTS

To illustrate the effect of differing GM and WM metabolite relaxation times, we show only the case of molal concentrations, as the term $R_{GM,WM}$ is a scaling factor that will affect the molarity and molality expressions similarly. To this end, the GM–WM-averaged $T_1$ and $T_2$ metabolite times in Equation 11 were replaced by the tissue-specific times reported in Mlynarik et al.19 ($T_{1GM, NAA} = 1.47 \text{ s}$, $T_{1WM, NAA} = 1.35 \text{ s}$, $T_{2GM, NAA} = 247 \text{ ms}$, $T_{2WM, NAA} = 295 \text{ ms}$). Data were simulated for five sets of TR and TE times: TE/TR = 0.006 s/3 s, TE/TR = 0.006 s/2 s, TE/TR = 0.006 s/1.5 s, TE/TR = 0.020 s/1.5 s and TE/TR = 0.040 s/1.5 s. For comparison, data were also simulated with a single averaged $T_1$ and $T_2$ for both GM and WM. The plots of these data are shown in Figure 1. Along the bold line are overlaid the plots of data corrected with Equation 11 using the correct relaxation times across the range of TE and TR, yielding the correct results in each case. The thicker lines that deviate from the bold line illustrate the errors that arise when using the same averaged relaxation times in Equation 11 for any of the tested TE and TR combinations (i.e. lines for all five sets overlap). The thinner lines show increasing deviations from the thick (correct) line with increasing TE or decreasing TR (i.e. maximum deviation with TE/TR = 0.04 s/1.5 s; minimum deviation with TE/TR = 0.006 s/3.0 s).

Estimation of the absolute metabolite concentrations while accounting for compartment-specific GM and WM signal relaxation times requires an assumption about the GM/WM metabolite ratio in order to assign each fraction of the total metabolite signal its distinct relaxation factor. The errors in the concentration estimates will depend not only on the error in this ratio, but on the tissue composition and assumed relaxation times. The error will be zero for voxels containing only WM or only GM and CSF, and will be maximal at an intermediate mix of GM and WM. To examine the errors for a representative case, data simulated as above, with TE = 40 ms, TR = 1.5 s and a metabolite GM/WM ratio of 1.2 (18/15), were analyzed assuming an ‘incorrect’ ratio of 1.5 (25% greater than the correct ratio). For this case, the maximum error was less than 0.5%.
Illustrated in Figure 2A is the application of Equation 11 to NAA measurements obtained from a healthy human subject with TE = 40 ms and TR = 1.5 s and corrected using the averaged $T_1$ and $T_2$ values for GM and WM used in the simulations.

In Figure 2B, the same raw data are plotted after correction with Equation 11 assuming different metabolite signal $T_1$ and $T_2$ values in GM and WM (the same values as used in the simulation) and a GM/WM NAA concentration ratio of 1.3. The latter factor was derived from the regression involving the data corrected with the same averaged $T_1$ and $T_2$ times for both GM and WM (shown in Figure 2A). Notable are the lower WM and higher GM extrapolated endpoints of the regression relative to the endpoints of the regression involving the molal data shown in Figure 2A. The ‘100% WM’ endpoint is 3.7% lower and the ‘100% GM’ endpoint is 3.8% higher than these values when assuming single averaged $T_1$ and $T_2$ values in both tissues, resulting in a higher GM/WM metabolite concentration ratio estimate of 1.4. This is similar to the results obtained in the simulated data analysis when the data were generated with TE = 40 ms and TR = 1.5 s, and tissue-specific relaxation times were used rather than tissue-averaged times (bold line versus thin line for TE/TR = 0.04/1.5 s in Figure 1). The sum of the square residuals of the linear regression shown in Figure 2A was within 0.1% of the sum of the square residuals of the linear regression shown in Figure 2B. Hence, no improvement in the fit of the data to a straight line was observed when using tissue-specific relaxation factors to adjust the data in this case. To explore the possibility of converging towards a stable value for the metabolite ratio with an iterative application of Equation 11, the analysis was repeated assuming a GM/WM metabolite ratio of 1.4, but without a substantial change (<0.1%) in the results.

5 DISCUSSION

The reliable estimation of neurometabolite concentrations by $^1$H-MRS faces numerous challenges, not least of which is the heterogeneity of the tissue within the voxel. To simplify this problem, it is generally assumed that the sampled brain region is composed of only three compartments – GM, WM and CSF – and, at times, a fourth compartment encompassing an MRI-detectable lesion. Each of these compartments is likely to have not only a different metabolite concentration and water density, but also different relaxation times associated with the signals. In a previous report, we presented Equation 2 for adjusting the molal concentration estimates for partial volume and water signal relaxation effects. We also examined the impact of errors arising from estimates of the relative fractions of GM, WM and CSF in the voxel (segmentation errors), as well as errors in the water signal relaxation times associated with these fractions. In the present article, we extend Equation 2, together with a similar equation for the estimation of molarity, to account for tissue-specific metabolite signal relaxation. To our knowledge, this is the first valid expression presented to accomplish this. In view of the alternative methods that have been proposed for the performance of this correction, it is
worth emphasizing that scaling only the GM and WM volume or water fractions by the distinct metabolite relaxation factors is not equivalent to scaling the GM and WM metabolite signal fractions by these factors. In order to calculate the size of the latter signal fractions, the relative metabolite concentrations in GM and WM must be known or assumed. Furthermore, any valid expression for the correction of metabolite signal relaxation differences must reduce to the simpler valid equations under simplifying conditions, such as when the observed signals are fully relaxed or when there is only a single tissue type in the voxel.

We analyzed the case of just one metabolite (NAA) at a field strength of 3 T in this work, assuming relaxation times previously reported for this metabolite at 3 T. These results, of course, can be extended to any metabolite under different sets of assumptions with respect to partial volume fractions, tissue concentrations, relaxation rates and pulse parameters: the general trends in errors resulting from failing to take into account differences in tissue-specific relaxation rates, or assuming inaccurate values, will be the same. As the principal factor underlying these errors will be the difference in GM and WM metabolite relaxation times, a knowledge of these times is essential to correct for them accurately.

Unfortunately, such information is lacking for most metabolites across the wide range of magnetic field strengths currently in use for brain studies, and few studies have attempted to measure the relaxation times of the various distinct proton signals that make up a metabolite spectrum. Adding to this uncertainty, metabolite relaxation times have been shown to vary with age, pathology and brain region and, given that metabolite errors will be the difference in GM and WM metabolite relaxation times, a knowledge of these times is essential to correct for them accurately.

In summary, the brain tissue milieu sampled by a typical MRS voxel is complex, conferring different MR properties on the detected signals, and could be predicted based on the differing GM and WM relaxation rates alone. However, in voxels with mixed tissue composition, the full expression (Equation 11) is needed to estimate the effects of tissue-specific relaxation. This entails that the relative metabolite concentrations in each compartment are assumed, as, when dealing with real data, they are not known a priori. Reasonable assumptions about GM and WM metabolite concentrations may be obtained from previous estimates, for example, from multi-voxel studies in which the fractional signals are assumed to relax with common T1 and T2 times. In this present article, we adjusted NAA spectroscopic imaging data with different GM and WM NAA signal relaxation times using Equation 11 and a GM/WM NAA ratio obtained from a previous analysis in which equivalent tissue-averaged GM and WM relaxation times were assumed. Repeating the tissue-specific analysis with the concentration ratio obtained in the previous analysis did not substantially alter the results (<0.1%). We note that this approach would not be feasible in single-voxel studies, and hence assumptions about the GM/WM concentration ratio for any metabolite would be an important caveat in the interpretation of the results, particularly in studies on pathological tissue or differing regions of the brain. Although the errors in GM or WM metabolite concentration estimates are small in the simulated and real data analyses of this report, they nonetheless introduce a measurable analysis bias in the results.

To address the issue of tissue-specific water signal relaxation, a method has been proposed to measure brain water signal relaxation and density differences on a voxel-by-voxel basis by acquiring in situ water relaxation and density maps together with 1H-MRS imaging data. However, although reliable measurement of the relaxation rates of the large water signal can be accomplished with straightforward imaging techniques, in situ GM and WM metabolite signal relaxation measurements are prohibitively time consuming, technically challenging and hence impractical in a clinical setting. Given this impracticality, the most straightforward approach to improve the accuracy of absolute concentration estimates is simply to use acquisition parameters that minimize the impact of signal relaxation as much as possible, a remedy that has long been recommended to improve the accuracy of MRS concentration estimates. The minimization of T2 effects has become feasible with the advent of ultrashort-TE sequences, whereas the acquisition of the water signal with a small pulse angle reduces the impact of water signal T1 differences. Small pulse angles, unfortunately, are not practical for the acquisition of metabolite signals, and lengthening TR comes at a cost of increasing the total acquisition time. For the example of the NAA signal and assumed relaxation times at 3 T used in this report, we estimate the errors to be as large as 8% when TE = 144 ms and TR = 1.5 s, but less than 1% when tissue-averaged rather than tissue-specific relaxations are used for partial volume correction of data acquired with TE = 6 ms and TR = 6 s.

6 CONCLUSION

In summary, the brain tissue milieu sampled by a typical MRS voxel is complex, conferring different MR properties on the detected signals, depending on the cellular or extracellular compartment of origin. In order to interpret these signals in a practical manner, simplifying assumptions need to be made. In this article, we present an extension of our earlier expression for the correction of partial volume effects in 1H-MRS data from the brain assuming three compartments: GM, WM and CSF. The fuller expression accounts for tissue-specific metabolite signal relaxation and requires assumptions not only with respect to the relaxation times, but with respect to the relative metabolite concentrations in GM and WM. With simulated data, we demonstrate the magnitude of the errors that arise when there are differences in GM and WM metabolite signal relaxation times that are not taken into account, or when incorrect assumptions about the GM/WM concentration ratio are made. Given the substantial technical challenges of reliable measurement of metabolite signal relaxation in situ, the simplest approach to reducing these errors is to acquire the data with pulse sequence parameters that minimize the effect of relaxation on the signal.

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REFERENCES

1. Hetherington HP, Pan JW, Mason GF, et al. Quantitative H-1 spectroscopic imaging of human brain at 4.1 T using image segmentation. Magn Reson Med. 1996;36(1):21-29.

2. Tal A, Kirov II, Grossman RL, Gonen O. The role of gray and white matter segmentation in quantitative proton MR spectroscopic imaging. NMR Biomed. 2012;25(12):1392-1400.

3. Mandl RC, van den Heuvel MP, Klop DW, et al. Tract-based magnetic resonance spectroscopy of the cingulum bundles at 7 T. Hum Brain Mapp. 2012;33(7):1503-1511.

4. Nuzzillard D, Bourg S, Nuzzillard J. Model-free analysis of mixtures by NMR using blind source separation. J Magn Reson. 1998;133(2):358-363.

5. Ladrour C, Howe FA, Griffiths JR, Tate AR. Independent component analysis for automated decomposed of in vivo magnetic resonance spectra. Magn Reson Med. 2003;50(4):697-703.

6. Sajda P, Du S, Brown TR, et al. Nonnegative matrix factorization for rapid recovery of constituent spectra in magnetic resonance chemical shift imaging of the brain. IEEE Trans Med Imaging. 2004;23(12):1453-1465.

7. Lee P, Adany P, Choi IV. Imaging based magnetic resonance spectroscopy (MRS) localization for quantitative neurochemical analysis and cerebral metabolism studies. Anal Biochem. 2017;529:40-47.

8. Harris AD, Saleh MG, Edden RA. Edited 1H magnetic resonance spectroscopy in vivo: methods and metabolites. Magn Reson Med. 2017;77(4):1377-1389.

9. Ernst T, Kreis R, Ross BD. Absolute quantification of water and metabolites in the human brain. I. compartments and water. J Magn Reson. 1993;102:1-8.

10. Barker PB, Soher BJ, Blackband SJ, Chatham JC, Mathews VP, Bryan RN. Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. NMR Biomed. 1993;6(1):89-94.

11. Michaelis T, Merboldt KD, Bruhn H, Hanicke W, Frahm J. Absolute concentrations of metabolites in a localized proton MR spectra. Radiology. 1993;187(1):219-227.

12. Christiansen P, Henriksen O, Stubaard M, Gideon P, Larsson HB. In vivo quantification of brain metabolites by 1H-MRS using water as an internal standard. Magn Reson Imaging. 1993;11(1):107-118.

13. Gasparovic C, Song T, Devier D, et al. Use of tissue water as a concentration reference for proton spectroscopic imaging. Magn Reson Med. 2006;55(6):1219-1226.

14. Knight-Scott J, Haley AP, Rossmiller SR, et al. Molality as a unit of measure for expressing 1H MRS brain metabolite concentrations in vivo. Magn Reson Imaging. 2003;21(7):787-797.

15. Di Costanzo A, Trojsi F, Tosetti M, et al. Proton MR spectroscopy of the brain at 3 T: an update. NMR Biomed. 2012;6(5):321-333.

16. Rupsingh R, Borrie M, Smith M, Wells JL, Bartha R. Reduced hippocampal glutamate in Alzheimer disease. Neurobiol Aging. 2011;32(5):802-810.

17. Gasparovic C, Bedrick EJ, Mayer AR, et al. Test-retest reliability and reproducibility of short-echo-time spectroscopic imaging of human brain at 3T. Magn Reson Med. 2011;66(2):324-332.

18. Mlynarik V, Gruber S, Moser E. Proton T (1) and T (2) relaxation times of human brain metabolites at 3 Tesla. Magn Reson Med. 1996;36(1):21-29.

19. Mlynarik V, Adany P, Choi IY. Imaging based magnetic resonance spectroscopy (MRS) localization for quantitative neurochemical analysis and cerebral metabolism studies. Anal Biochem. 2017;529:40-47.

20. Tal A, Kirov II, Grossman RL, Gonen O. The role of gray and white matter segmentation in quantitative proton MR spectroscopic imaging. NMR Biomed. 2012;25(12):1392-1400.

21. Mandl RC, van den Heuvel MP, Klop DW, et al. Tract-based magnetic resonance spectroscopy of the cingulum bundles at 7 T. Hum Brain Mapp. 2012;33(7):1503-1511.

22. Nuzzillard D, Bourg S, Nuzzillard J. Model-free analysis of mixtures by NMR using blind source separation. J Magn Reson. 1998;133(2):358-363.
33. Knight-Scott J, Shanbhag DD, Dunham SA. A phase rotation scheme for achieving very short echo times with localized stimulated echo spectroscopy. Magn Reson Imaging. 2005;23(8):871-876.

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