CEREBRAL METABOLITE AND WATER RELAXATION TIMES MEASURED BY INTRA-SCAN PROTON MAGNETIC RESONANCE SPECTROSCOPY AT 3.0 TESLA

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ABSTRACT
Absolute quantitative Proton MR Spectroscopy using cerebral water as a reference requires correction of the water signal for relaxation effects. Using the reference water signal collected at the beginning of the spectroscopy pulse sequence, this study suggests and implements a post-processing technique of estimating the relaxation times of cerebral metabolites and water acquired in a single data set, to circumvent the usual practice of prolonged acquisition of their signals in separate scans. Metabolite and water relaxation times were measured in the anterior cingulate cortex (ACC) and bilateral hippocampi of different healthy volunteer groups using standard saturation recovery and spin echo techniques, respectively. Experiments were conducted on a 3.0 T GE MR scanner, using the PRESS sequence with CHESS water suppression. The acquired spectra were separated into metabolite and water lines to estimate their respective peak areas using the SAGE software package (version 7). Monoexponential saturation and decay curves were then fitted to the peak areas to estimate the T_1 and T_2 relaxation times, respectively. T_1 estimates (mean ± SE, ms) in the ACC were: 1199 ± 363 (NAA), 1266 ± 167 (Cr), 1527 ± 286 (Cho), and 1574 ± 61 (water); while the T_2 estimates (mean ± SE, ms) were: 212 ± 28 (NAA), 148 ± 23 (Cr), 208 ± 24 (Cho), and 147 ± 6 (water). Water and metabolite relaxation times did not vary significantly between the left and right hippocampi ($p > 0.05$). The estimates reported here agreed with previous reports, thus confirming the accuracy of the technique.

INTRODUCTION
Magnetic resonance spectroscopy (MRS) provides a means of noninvasively interrogating tissue metabolism (Staffen et al., 2005; Tofts and Waldman, 2003), and is predominantly used in studies of brain disorders. MRS brain studies of mood disorders and inflammation mostly focus on the mood-regulating regions, such as the frontal brain (which includes the anterior cingulate cortex, ACC) and hippocampus (Davanzo et al., 2001; Drevets, 2007; Ham et al., 2007; Harrison et al., 2009; Mervaala et al., 2000; Moore et al., 1999; Moore et al., 2000; Taylor et al., 2009). The frontal brain in particular may be selected sometimes also due to the convenience of voxel placement in that region; the probability of acquiring good quality spectra from the frontal brain is also high if the voxel is well placed away from the sinus.

Depending on the choice of echo time (TE) and repetition time (TR) for spectral acquisition, MR spectra could suffer significant signal losses (of between 14 % and 50 % (Drost et al., 2002;

Tofts and Waldman, 2003)) through longitudinal (T_1) and transverse (T_2) relaxation processes. Absolute spectral quantification will then usually require correction for these relaxation effects, using relaxation times either from the literature (Brief et al., 2009; Dixon et al., 2002) or measured from the regions of interest within the same study (Barker et al., 1993; Hennig et al., 1992; Wu et al., 2005), the former being the choice of most studies due to scan time considerations particularly if the water signal is of interest. In the latter case, the scan duration is usually prolonged as spectra would have to be measured at varying TEs and TRs. Where the relaxation time of cerebral water signal is required, the TE and TR ranges will have to be wider than those that would normally be used for only the cerebral metabolites (Brief et al., 2009).

Ideally, relaxation effects can be kept to a minimum if $TE \approx 0$ ms and $TR \approx 5$ x the T_1 of the signal of interest. In other words, a short TE and a long TR (theoretically $TE = 0$, $TR = \infty$) should be used for minimal signal losses arising from relaxation effects (Drost et al., 2002; Tofts and Waldman, 2003). However, since magnetic field gradients must be applied to define the spectroscopic voxel, TE can never be zero, and because the sequence has to be repeated many times, a TR of infinity is not practical because the patient cannot be scanned for too long a time. A well planned TE/TR pair is thus required in order to obtain spectra that exhibit reasonable T_1 and T_2 relaxation effects. This therefore implies that the need for correction for relaxation effects (either T_1 or T_2, or both) still remains if the aim of the study is to accurately measure metabolite concentrations.

The measured or literature relaxation times are normally substituted into MRS sequence-specific exponential equations to yield the required correction factors. These correction factors are then used in a study-specific absolute quantification model equation to estimate the metabolite concentrations in molar units (not done in this study). All studies (Barker et al., 1993; Brief et al., 2009; Dixon et al., 2002; Hennig et al., 1992; Wu et al., 2005) conducting absolute metabolite quantification basically use these steps but the challenge that remains is the accurate measurement of the metabolite and water relaxation times, in the same study within patient tolerant time limits. Clearly, the approaches adopted by those studies are not suitable for clinical spectroscopy (Brief et al., 2009) due to scan time considerations.

This paper is therefore targeted at clinical spectroscopy for absolute metabolite and water quantification, by presenting a post-processing method that can be used to estimate the relaxation times of water and metabolites. The technique optimizes the MRS data at a given TE/TR pair by first separating the reference or unsuppressed -water signal (acquired by default by the MR scanner) from the metabolite or water-suppressed signal, where both data segments have been acquired by the MR scanner simultaneously within the same TE/TR time.

By this method, the MRS acquisition protocol for a given study is also included in the TE/TR set used in the measurement of the relaxation times; there is also no need for turning off the water suppression module (e.g. CHESS) on the MR scanner to record the water signal. The overall advantage of the technique is that the total data acquisition time is reduced to at least half the expected time.

The main objective of this study therefore was to optimize a series of MRS acquisitions in order to simultaneously measure the relaxation times of cerebral metabolites and water in the frontal (ACC) and bilateral hippocampal brain regions. The aim was to provide T_1 and T_2 relaxation time estimates for the major metabolite and water signals for future absolute quantitative MRS studies focusing on the prefrontal and hippocampal brain areas, especially for MRS studies in psychiatry and inflammation (both peripheral, e.g. arthritis, and central nervous disorders, e.g. multiple sclerosis). The spectral processing technique presented will also serve a good purpose to quantification schemes that use the endogenous water signal as reference. The major cerebral metabolites reported for the left and right

hippocampi were N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), and myo-inositol (mI). In addition to these major metabolites, glutamate (Glu) and glutamine (Gln) were also measured in the anterior cingulate cortex (ACC).

METHOD

Subjects

Following ethical approval of the study by the West of Scotland Research Ethics Committee 4 (WoSREC4), a total of eighteen healthy subjects (10 males, 8 females) were studied for T_1 and T_2 relaxation times of the metabolites and water. Each volunteer gave prior informed written consent. No volunteer had any neurological or psychiatric disorder. Volunteers were randomly assigned to four relaxation time measurement experiments as follows:

EXPERIMENT 1: T_1 in the ACC (Fig. 1), involving three (1 M/2 F) volunteers aged 27-55 years (mean age = 34.3 years);

EXPERIMENT 2: T_2 in the ACC, involving seven (3 M/4 F) volunteers aged 27-60 years (mean age = 33.4 years);

EXPERIMENT 3: T_1 in the bilateral hippocampi (Fig. 2a-b), involving three (2 M/1 F) volunteers aged 25-33 years (mean age = 22.3 years); and

EXPERIMENT 4: T_2 in the bilateral hippocampi, involving four (3 M/1 F) volunteers aged 27-53 years (mean age = 34.6 years).

MR Imaging

MR imaging and spectroscopy acquisitions were performed on a 3.0 T GE Signa HD MRI/MRS scanner (software version 12.5; Milwaukee, WI, USA) equipped with an eight-channel receive-only head coil.

In planning the MRS voxels, two structural scans of the brain were conducted on each subject: a sagittal (Fig. 1) T_1-weighted FLAIR ($TE/TR/TI$ = 9/2685/920 ms) and an axial (Fig. 2a-b) T_2-weighted FSE/propeller (TE/TR = 110/5000 ms) MRI. For the sagittal scan, 21 slices (each of thickness 4 mm) were planned along the axes of the hippocampi. For the axial scan, 22 oblique slices (each of thickness 5 mm) were planned parallel to the long axis of the hippocampus. In both structural scans, a field of view of 24.0 x 24.0 cm^2 was chosen, with an in-plane image matrix of 512 x 512 voxels to give good image resolution. The T_2-weighted FSE/propeller sequence produced images with good gray/white matter contrast.

MRS Protocol

For both T_1 and T_2 studies, the same MRS acquisition protocol was maintained. This involved the acquisition of 128 averages (i.e. NSA = 128) of CHESS water-suppressed spectra from the prefrontal gray matter or ACC (Fig. 1) and the bilateral hippocampi (Fig. 2a-b). Voxel sizes of 2.0 x 3.0 x 2.0 cm^3 and 3.5 x 1.2 x 1.0 cm^3 (corresponding to the AP x LR x SI directions)

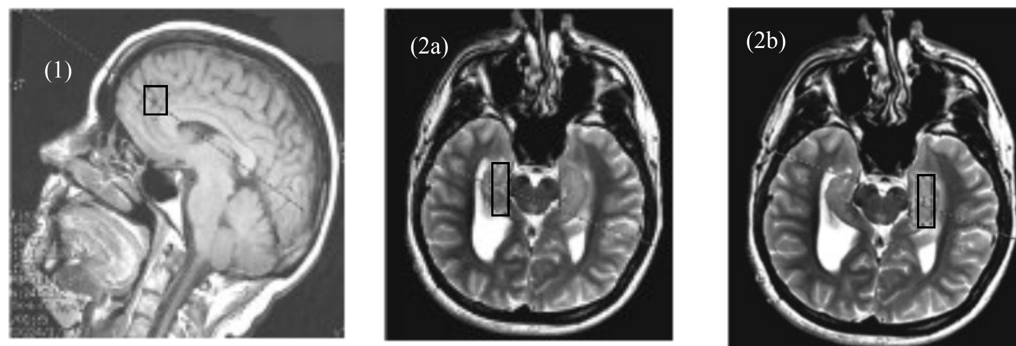

Fig. 1-2: Voxel placements in the ACC (1), right (2a) and left (2b) hippocampi

were used for the prefrontal and hippocampal regions, respectively. For the acquisitions in the bilateral hippocampi, the same voxel was moved from the right to left hippocampus after recording spectra from the former, to ensure consistency in the size of the voxel placed in the two brain hemispheres. The following parameters were maintained in all spectral acquisitions: NEX = 8, bandwidth = 5000 Hz, and 4096 data points.

Measurements of Metabolite and Water T_1 Relaxation Times

T_1 measurements were carried out using the saturation recovery method with a variable *TR* PRESS localisation sequence. A constant *TE* value of 23.0 ms was maintained, while single voxel spectra were acquired at six *TR* times: 1070, 1200, 1500, 2500, 3000 and 3500 ms.

Measurements of Metabolite and Water T_2 Relaxation Times

T_2 measurements were carried out using a multi spin-echo PRESS localization pulse sequence with a fixed *TR* value of 2000 ms. Spectra were acquired sequentially with *TE* values of 35, 85, 128, 164, 262, 326, 400 and 600 ms.

Spectral Processing and Quantification of Relaxation Times

All spectra were processed and quantified using the SAGE software package (version 7; GE Healthcare, Little Chalfont, Buckinghamshire, UK), a dedicated semi-automated spectral fitting program. The MRS data (N_{Total}) is a combination of water (16/NEX) and metabolite (NSA/NEX) spectra given by:

$$N_{Total} = 16/NEX + NSA/NEX \quad (1)$$

Since the water spectra are acquired from the same voxel as the metabolite spectra, both signals should be affected by the same factors such as effects of eddy currents, motion and relaxation (Klose, 1990; Natt et al., 2005; Provencher, 1993). Therefore, using the same analysis procedure, it should be possible to estimate the T_1 and T_2 relaxation times for cerebral water and metabolites from the same data set. The spectral processing procedure described below was thus preceded by separating the water and metabolite signals according to equation 1: with NEX = 8 and NSA = 128, 2 water spectra and 16 metabolite spectra were averaged in all data sets.

Water and metabolite signals in the time domain were each corrected for eddy currents, followed by signal averaging from the eight channels of the head coil as described previously (Wright et al., 1997). The FIDs were then Fourier transformed, phase and baseline corrected, to yield the desired spectral profiles. Water and metabolite spectral areas were then quantified as described elsewhere (Mumuni, 2013).

Estimation of T_1 Relaxation Times

Estimates of the metabolite and water T_1 relaxation times were obtained from the solution of a nonlinear least square monoexponential recovery fit of the quantified peak areas, $A_z(TR)$ at their respective *TR* values using the following equation for the PRESS sequence:

$$A_z(TR) = A_\infty \times [1 - \exp(-TR/T_1)] \quad (2)$$

where A_∞ is the spectral peak area at the longest possible *TR* time.

Estimation of T_2 Relaxation Times

Estimates of the metabolite and water T_2 relaxation times were obtained from the solution of a nonlinear least square monoexponential decay fit of the measured peak areas, $A(TE)$ at their respective *TE* values using the following equation for the PRESS sequence:

$$A(TE) = A_o \exp(-TE/T_2) \quad (3)$$

where A_o is the maximum spectral peak area at $TE \approx 0$ ms.

The curve fitting routines for the estimation of both T_1 and T_2 relaxation times were performed using the curve fitting toolbox in MATLAB (version 7.8.0.347, R2009a; Simulink, Natick, Massachusetts, USA).

Statistical Analysis

Only the relaxation times measured in the left

and right hippocampal regions were compared using the paired t-test, assuming the data was normally distributed. Statistical tests were performed using the Minitab software package (version 16, Minitab Inc., State College, Pennsylvania, USA). $P < 0.05$ was considered a significant difference in the comparisons.

RESULTS

Tables 1 and 2 summarize the mean relaxation times of the metabolites and water measured in the ACC (Fig. 1) and bilateral hippocampi (Fig. 2a-b), respectively. The uncertainties of all measurements are given in terms of the standard error (SE).

The brain sites studied were predominantly gray matter (GM) regions. For the ACC, the voxel was placed centrally across the two hemispheres and so the relaxation time estimates were averages from both hemispheres. Water and all the metabolites exhibited similar T_1 and T_2 relaxation time patterns in the three regions studied (Tables 1 and 2).

T_2 relaxation times for both left and right hippocampal myo-inositol (mI) could not be estimated as the fits were unreliable; these were therefore removed from the analysis (Table 2). Relaxation time differences between the left versus right hippocampi are indicated by the corresponding p -values of the paired t-tests (Table 2). The paired t-tests did not show significant T_1 and T_2 relaxation time differences between the left and right hippocampi (i.e., $p > 0.05$ in all cases). Since the sample sizes were small for both T_1 and T_2 experiments, the Wilcoxon singed-rank test was further used (assuming non-normal distribution of the data) in order to see if the comparisons will yield a different outcome. It was necessary to do this as the data was not originally tested for normality. However, no significant difference in relaxation times was shown between the two hippocampi in this analysis also.

Variation in the relaxation time estimates among the ACC, left and right hippocampi was not assessed statistically since the measurements were conducted in different groups of volunteers, whose characteristics by themselves could contribute some variability in the measurements.

Table 1: Average relaxation times of metabolites and water in the prefrontal gray matter (ACC) region

Peak	T_1 (ms, ± SE) $n = 3$	T_2 (ms, ± SE) $n = 7$
NAA	1199 (363)	212 (28)
Glu	1193 (265)	62 (7)
Gln	1436 (131)	56 (10)
Cr	1266 (167)	148 (23)
Cho	1527 (286)	208 (24)
mI	1368 (262)	125 (21)
Water	1574 (61)	147 (6)

Table 2: Average relaxation times of metabolites and water in the bilateral hippocampi

Peak	T_1 (ms, ± SE); $n = 3$			T_2 (ms, ± SE); $n = 4$		
	Right	Left	Comparison (p-value)	Right	Left	Comparison (p-value)
NAA	1283 (472)	1547 (119)	0.91	114 (32)	153 (43)	0.49
Cr	1131 (337)	1144 (232)	0.96	113 (28)	105 (16)	0.86
Cho	1414 (206)	1365 (207)	0.98	251 (70)	338 (101)	0.53
mI	1462 (442)	1772 (797)	0.61	-	-	-
Water	1389 (58)	1475 (68)	0.53	273 (98)	178 (83)	0.23

Ideally, such an assessment could have been conducted if each group of measurements (i.e., T_1 and T_2 relaxation times) were carried out in the same individual volunteers. Nonetheless, from the graphical representations of the T_1 (Fig. 3) and T_2 (Fig. 4) time estimates for these three regions, while metabolite relaxation times (except T_2 of NAA) do not seem to vary significantly across the three brain regions, relaxation times for water appear to vary between the ACC and the right hippocampus.

Even though the comparisons shown in Figures 3 and 4 were not subjected to any statistical test, the plots indicate some difference in the T_1 relaxation time of water in the ACC and right hippocampus (Fig. 3); T_2 relaxation times of water

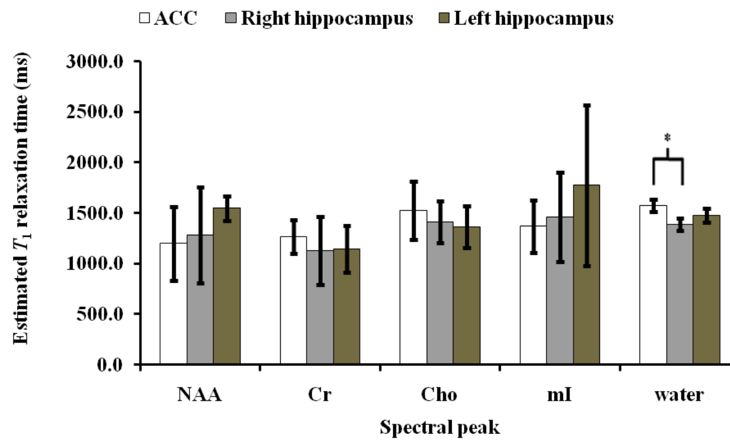

Fig. 3. Measured T_1 relaxation times for the major metabolites and water compared between the ACC, left and right hippocampi. Error bars represent standard error of T_1 estimates. *Significantly different

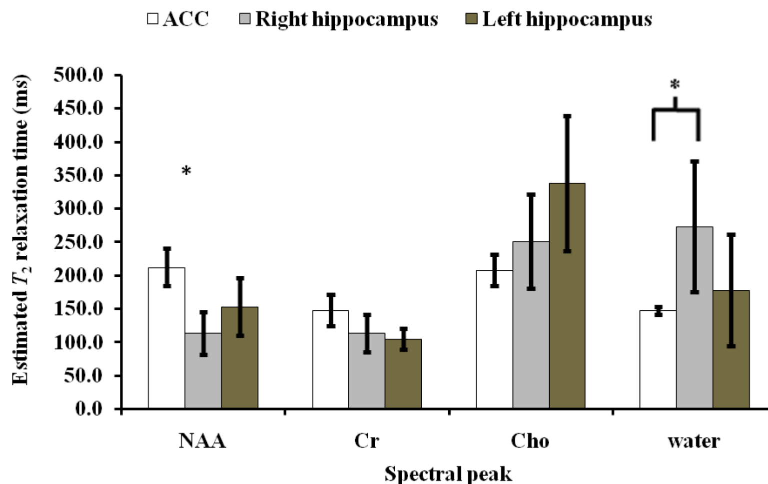

Fig. 4. Measured T_2 relaxation times for the major metabolites and water compared between the ACC, left and right hippocampi. Error bars represent standard error of T_2 estimates. *Significantly different

and NAA were also significantly different between the ACC and right hippocampus (Fig. 4). Apart from these two spectral peaks in the stated regions, no other comparison showed any significant difference. Estimates for the ACC and left hippocampus were however not too different.

DISCUSSION

Generally, T_2 estimates were only about a tenth of the T_1 estimates, consistent with theoretical predictions. However, T_2 relaxation times for Glu and Gln were lesser than their T_1 relaxation times by a factor of one hundred (Table 1), sharply deviating from what is expected theoretically. This could have been due to reasons including, but not limited to, coupling between the two spectra, poor spectral resolution, and substantial decay of the two spectra within the TE range used for the measurements. The estimated T_1 and T_2 relaxation times did not generally vary significantly among the three brain regions since those regions were all predominantly the same tissue type (i.e., gray matter). Both T_1 and T_2 were not also significantly different between the left and right hippocampi, indicating an approximate equality of relaxation times between contralateral sections of the brain. However, left hippocampal T_1 estimates showed some tendency toward higher values than those estimated from the right hippocampus. Relaxation times varied substantially between the ACC and right (but not left) hippocampus. This was unexpected and could have thus been due to random error, especially when laterality was established between the left and right hippocampal measurements. Variation in regional brain relaxation times was not explored statistically because the data was recorded from different groups of healthy volunteers. Both T_1 and T_2 relaxation times for the regions studied were in agreement with published results presented in the following discussion.

With a similar spectral acquisition protocol to the one reported in this study, T_2 relaxation times of NAA, Cr and Cho in the anterior cingulate gyrus GM were found to be 254 (15), 161 (10) and 242 (37) ms respectively in six healthy subjects at 3.0 T (Träber et al., 2004). However, in reporting their T_1 values, averages from the occipital WM, motor cortex and fronto-lateral WM/GM were provided (Träber et al., 2004): NAA, Cr, Cho and mI exhibited 1340 (80), 1110 (110), 1140 (70) and 980 (160) ms, respectively; where values in parenthesis are standard deviations in all cases. Pure GM T_1 values (in the occipital region) have also been reported elsewhere (Mlynárik et al., 2001) at 3.0 T: NAA, Glu, Cr, Cho and mI had 1470 (120), 1270 (100), 1460 (70), 1300 (60) and 1230 (90) ms, respectively; where values in parenthesis are standard errors. However, relaxation times for Gln and water in the prefrontal gray matter region (ACC) have not been reported in the literature to the best of the authors' knowledge.

From STEAM single-voxel experiments at 1.5 T (Brief et al., 2003), frontal white matter metabolites' T_1 have been measured to be 1420 (70), 1770 (80) and 1420 (70) ms for NAA, Cr and Cho, respectively. Using the PRESS localization sequence at 1.5 T (Brief et al., 2005), T_2 values of NAA, Cr and Cho in occipital GM were estimated to be 256 (6), 159 (8), and 249 (7) ms, respectively; where values in parenthesis are standard errors in both studies.

With the STEAM sequence at 2.0 T, the relaxation times of NAA, Cr, Cho and mI were measured and averaged in the bilateral hippocampi of eight healthy subjects (Choi and Frahm, 1999). The average hippocampal T_1 relaxation times were: NAA = 1408 (253), Cr = 1554 (317), Cho = 1438 (365), mI = 1726 (512) ms; whereas the T_2 values were: NAA = 283 (62), Cr = 191 (43), Cho = 334 (106), mI = 138 (14) ms, where the values in parenthesis are standard deviations.

Cho and NAA consistently showed longer T_2 relaxation times than Cr as observed in this study and in the literature. Unlike brain tissue water T_1, metabolite T_1 relaxation times do not seem to depend on field strength, B_o (Mlynárik et al., 2001), which could be why the results reported here (at 3.0 T) are comparable to those at 1.5 T (Brief et al., 2003) and 2.0 T (Choi and Frahm, 1999). The reason for this non-

dependence of metabolite T_1 on B_o could be that the major metabolite peaks in the spectrum arise from non-exchangeable protons, and unlike water protons, they have reduced interactions with less mobile macromolecular protons (Mlynárik et al., 2001).

No study was found in the literature reporting the relaxation times of water in the prefrontal and hippocampal brain regions using MRS. However, an assumption of a monoexponential relaxation of tissue water protons could lead to underestimation of the T_1 and T_2 relaxation times reported here. Water protons in a healthy brain are reported to exist in three tissue compartments (Whittall et al., 1997), and so a monoexponential fit, as has been done here, may be inaccurate for some of the spectra considered. More sophisticated fits on the other hand would require the acquisition of more data points (Barker et al., 1993) than the numbers acquired in this study. Thus, given the number of data points acquired in this study, the monoexponential fit was appropriate.

Similar to most brain studies, the small sample size in each experiment was a limitation. However, care was taken to ensure that quality spectra were acquired from each volunteer, with particular attention to voxel size and placement and shimming efficiency. Thus, even though the uncertainties (expressed as standard errors) in the measurements were quite large due to the small sample sizes, the average relaxation times were still found to compare with literature values.

The use of the GE MR scanner and its accompanying dedicated spectral processing software package could be another limitation of this study. Attempt was not made to repeat the study on an MR scanner from a different manufacturer. However, the implementation scheme of the MRS pulse sequence is similar across the various types of clinical MR scanners and so post-processing the data in the manner presented here should be possible on an equivalent spectral processing software package (e.g. the java-based MR user interface for the processing of *in vivo* MR spectra, jMRUI (Naressi et al., 2001)).

Relaxation time studies are few, and vast differences in brain regions have been studied. It is therefore difficult to make direct comparisons with estimates in the literature, given that differences in study subjects and regions of interest could contribute to variances in the estimates. At least, the regions studied in this paper are the most common regions of interest in most neurological and psychiatric MRS studies. Thus, the estimates would be of use to researchers considering absolute quantitative MRS of these regions. The availability of these results would save them time, resources and problems with patient compliance issues. This is because there will be no need for patients to undergo prolonged scans to measure the metabolite and water spectra and their relaxation times within the same study.

CONCLUSION

Cerebral water and metabolite signals were acquired within the same MRS PRESS sequence, and their relaxation times were estimated separately in the prefrontal and bilateral hippocampal regions using a novel post-processing technique aided by the SAGE software package. The measured relaxation times were consistent with literature values and can thus be used as references to correct metabolite signals for relaxation effects within the brain regions considered in this paper. In brain disorders suspected to impact on metabolite relaxation times, the results reported here could also be used as reference values in order to evaluate the extent of change in relaxation times with disease. Lastly, the spectral processing technique presented could be applied in patient studies to obtain accurate results within tolerable scan times.

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