Effect of Carr-Purcell refocusing pulse trains on transverse relaxation times of metabolites in rat brain at 9.4 T

Dinesh Kumar Deelchand, **Pierre-Gilles Henry**, and **Małgorzata Marjańska**
Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, Minneapolis, MN 55455, USA

Abstract

Purpose—To investigate the effect of Carr-Purcell (CP) pulse trains on transverse relaxation times, T_2, of tissue water and metabolites (both non-coupled and *J*-coupled spins) in the rat brain at 9.4 T using LASER, CP-LASER and $T_{2\rho}$-LASER sequences.

Methods—Proton NMR spectra were measured in rat brain in vivo at 9.4 T. Spectra were acquired at multiple echo times ranging from 18 to 402 ms. All spectra were analyzed using LCModel with simulated basis sets. Signals of metabolites as a function of echo time were fitted using a mono-exponential function to determine their T_2 relaxation times.

Results—Measured T_2s for tissue water and all metabolites were significantly longer with CP-LASER and $T_{2\rho}$-LASER compared to LASER. The T_2 increased by a factor of ~1.3 for non-coupled and weakly coupled spins (e.g., *N*-acetylaspartate and total creatine) and by a factor of ~2 (e.g., glutamine and taurine) to ~4 (e.g., glutamate and *myo*-inositol) for strongly coupled spins.

Conclusion—Application of a CP pulse train results in a larger increase in T_2 relaxation times for strongly coupled spins than for non-coupled (singlet) and weakly coupled spins. This needs to be taken into account when correcting for T_2 relaxation in CP-like sequences such as LASER.

Keywords

CP pulse train; Brain; LASER; LCModel; T_2

INTRODUCTION

Localization by adiabatic selective refocusing (LASER) (1) and semi-LASER (2) sequences are widely used for in vivo single-voxel ^{1}H NMR spectroscopy (MRS) studies in both humans and animals (3-9). These sequences offer excellent localization performance and greatly reduced chemical-shift displacement error, as well as diminished sensitivity to B_1 inhomogeneity (i.e., adiabaticity).

The succession of multiple adiabatic full-passage (AFP) pulses in LASER and semi-LASER constitutes a Carr-Purcell (CP) train (10) which is known to minimize *J*-evolution for *J*-coupled metabolites (11). The time evolution due to *J*-modulation becomes negligible when

Proofs and reprint requests to: Dinesh Kumar Deelchand, Ph.D. Center for Magnetic Resonance Research University of Minnesota 2021 6th St SE Minneapolis, MN 55455, USA Phone: (1) 612-625-8097 Fax: (1) 612-626-2004 dinesh@cmrr.umn.edu.

$\tau_{cp} \cdot \sqrt{\delta^2+J^2} \ll 1$ where τ_{cp} is an interpulse delay (in s), *J* is the coupling constant (in Hz), and δ is the chemical shift difference (in Hz) between the coupled spins. The preservation of the spectral pattern for several *J*-coupled metabolites e.g., glutamate, glutamine and *myo*-inositol (mIns) has been demonstrated successfully at relatively long echo times (T_E) in the human brain using CP-like PRESS sequences at 1.5 T (12) and 2 T (13). CPRESS (T_E = 45 ms) was also shown to improve detection of mIns in patients with cognitive impairment compared to the standard PRESS sequence at 3 T (14).

In addition to minimizing *J*-modulation, CP pulse trains also refocus the effects of diffusion and chemical exchange (15), resulting in longer apparent transverse relaxation times (T_2) than with standard sequences such as STEAM or PRESS. Accordingly, an increase in the apparent T_2 of tissue water in the human brain was reported using CP-LASER at 4 T and 7 T compared to PRESS (5) and LASER (16). Similarly, an increase in apparent T_2s was reported for non-coupled spins of *N*-acetylaspartate (NAA) and total creatine (tCr) with CP-LASER compared to PRESS (5). The term, apparent T_2, refers to the observed decay constant of the transverse magnetization, irrespective of the physical processes that contribute to the signal decay. Henceforth, terms, apparent T_2, T_2, and transverse relaxation time, will be used interchangeably.

In spite of the increasing popularity of CP-like sequences such as CP-PRESS, LASER and semi-LASER, however, there have been very few studies of the effect of CP pulse trains on T_2s of brain metabolites other than singlets. One study reported T_2s of mIns and the multiplet of NAA in human brain with CP-PRESS at 1.5 T (12). In that study, the authors noted that T_2 of mIns with CP-PRESS was somewhat longer than previously published with PRESS or STEAM. However, no precise quantitative comparison was made between PRESS and CP-PRESS, and T_2s for *J*-coupled metabolites were limited to mIns and the multiplet of NAA. Therefore, the quantitative effect of CP trains on T_2s of other coupled metabolites, such as glutamate, glutamine or taurine, remains to be determined.

The aim of the present study was to investigate the effect of CP pulse trains on the T_2s of metabolites (both non-coupled and *J*-coupled spins) in the rat brain at 9.4 T using LASER, CP-LASER and $T_{2\rho}$-LASER sequences.

METHODS

Male Sprague-Dawley rats (*n* = 6) were studied using a 9.4 T horizontal-bore magnet (Magnex Scientific, Oxford, UK) interfaced to an Agilent DirectDrive console (Agilent Technologies, CA, USA). The magnet was equipped with a gradient insert capable of reaching 400 mT/m in 300 μs (Resonance Research, Inc., MA, USA). The animals were anesthetized with 1.5% isoflurane in a mixture of 50% O_2:50% N_2O and placed in a cradle with the head fixed with bite-bar and ear rods. The body temperature was monitored throughout the study with a rectal thermal sensor and maintained at 37°C using a hot water circulation. All studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Localized in vivo ^{1}H NMR spectra from the brain were measured using LASER, CP-LASER and $T_{2\rho}$-LASER sequences (Figure 1). A volume-of-interest (VOI) of 62.5 μL (5 × 2.5 × 5 mm^3) was positioned on the midline 2 mm posterior to bregma and 3 mm ventral using transverse and sagittal RARE (17) images (repetition time, T_R = 4 s, T_E = 20 ms, echo train length = 8, field-of-view = 3 × 3 cm^2, matrix size = 256 × 128, slice thickness = 1 mm, 10 slices) and included tissue from cortex, hippocampus, and striatum. For all NMR measurements, a home-built quadrature 400 MHz ^{1}H surface RF coil (two loops, 14 mm diameter each) was used. B_0 shimming in the defined VOI was performed using an adiabatic version of FAST(EST)MAP (18) which resulted in a water linewidth of 13 ± 1 Hz.

The LASER sequence consisted of a 4 ms nonselective adiabatic half-passage (AHP) pulse followed by three pairs of gradient selective AFP pulses of the HS*n* family (1.5 ms duration, *n* = 1, R = 25, (19)) for 3D-localization (Figure 1A). T_E was defined as the total time from the end of the AHP pulse to the start of signal acquisition. The minimum T_E was 18 ms (corresponding to τ_{cp} = 3 ms) due to additional time needed for slice selective and spoiling gradients. For T_2 measurements, T_E was increased by adding free precession delays evenly distributed around the six AFP pulses (1).

In CP-LASER (Figure 1B), T_E was increased by adding AFP pulses rather than free precession delays. τ_{cp} was kept at 3 ms as in LASER. No gradient spoilers were used during the CP pulse train, and the CP pulses were phase cycled according to the MLEV scheme (20).

The $T_{2\rho}$-LASER sequence (Figure 1C) was similar to CP-LASER except that there was no delay between the CP pulses such that τ_{cp} was 1.5 ms (equal to the duration of one AFP pulse).

For LASER, ^{1}H spectra were acquired with eight T_Es: 18, 25, 32, 46, 60, 100, 200 and 400 ms. For CP-LASER and $T_{2\rho}$-LASER, CP train lengths (n_{mlev}) of 8, 16, 32, 64 and 128 AFP pulses were used, corresponding to T_Es of 42, 66, 114, 210 and 402 ms for CP-LASER and 30, 42, 66, 114 and 210 ms for $T_{2\rho}$-LASER. These T_Es correspond to the minimum T_E used in LASER (i.e., 18 ms) plus the length of the CP pulse train (i.e., $n_{mlev} \times \tau_{cp}$).

Water suppression was achieved using VAPOR (21). Spectra were acquired with T_R of 4 s, a spectral width of 10 kHz and 10,000 data points. Each FID was individually saved for shot-to-shot B_0 frequency correction. A non-suppressed water spectrum at each T_E was also acquired for eddy current correction. For each pulse sequence, macromolecule spectra were acquired using the inversion-recovery technique (22) for all T_Es except for T_Es > 200 ms where macromolecule signal was negligible.

All in vivo ^{1}H spectra were analyzed using LCModel version 6.1-4A (Stephen Provencher Inc., ON, Canada (23)). No baseline correction, zero-filling or apodization functions were applied to the in vivo data prior to the analysis. The spectra were fitted over the 0.5 to 4.2 ppm range. The model basis spectra for the various T_Es used in this study were simulated using home-written programs based on density matrix formalism in Matlab (The MathWorks, Inc., Natick, MA, USA) (24) using measured and published chemical shifts and *J*-coupling values (25). The spin evolution occurring during the adiabatic RF pulses was also

taken into account in the simulations. Separate basis spectra were generated for the singlet and multiplet signals of NAA, and the CH_2 and CH_3 groups of creatine and phosphocreatine (6). The signal from the methylene protons of tCr present in the macromolecule spectra (due to their short T_1 relaxation time) was removed in post-processing using HSVD in Matlab.

The T_2 values were determined by fitting the metabolite signals obtained from LCModel analysis as a function of T_E using a two-parameter, mono-exponential decaying function in Matlab. Only metabolites with Cramér-Rao Lower bounds (CRLBs) < 50% at all T_Es were considered (supplementary material), and T_2 values are reported for metabolites for which fits with goodness of fit (R^2) above 0.88 were obtained. The measured transverse relaxation times for each sequence are denoted by T_{2f} (free precession), $T_2^{\dagger}$ and $T_{2\rho}$ for LASER, CP-LASER and T_{2p}-LASER, respectively. The two-tailed, unpaired student's t-test was used to compare T_2s between pulse sequences.

RESULTS

Spectral appearance

In vivo ^{1}H NMR spectra acquired at various T_Es with LASER, CP-LASER and $T_{2\rho}$-LASER sequences in the rat brain are shown in Figure 2. In LASER, singlets showed signal reduction with increasing T_E due to T_2 relaxation while *J*-coupled metabolites underwent both *J*-evolution and T_2 relaxation, which is apparent mainly in the 2.1 to 2.5 ppm (glutamate and glutamine) and 3.4 to 3.6 ppm (mIns) regions. In CP-LASER and $T_{2\rho}$-LASER spectra, in contrast, the intensities of these multiplets were preserved at relatively long T_Es: glutamate and glutamine resonances were still visible at 114 ms while mIns began to undergo noticeable *J*-modulation only around 210 ms.

Spectra obtained with CP-LASER and $T_{2\rho}$-LASER were remarkably similar at the same T_E (Figure 2). However, small differences could be observed especially at long T_Es (Figure 3). For example, the signal intensity of mIns at T_E of 210 ms was lower with CP-LASER than with $T_{2\rho}$-LASER despite having very similar spectral pattern (Figure 3a). In addition, glutamate exhibited different *J*-modulation with CP-LASER than $T_{2\rho}$-LASER, and its intensity was higher with $T_{2\rho}$-LASER. At T_E of 402 ms, no mIns signal was observed with CP-LASER due to the *J*-modulation while under $T_{2\rho}$-LASER mIns did not undergo any *J*-evolution (Figure 3b). These differences can be attributed to the different number of CP pulses and the different τ_{cp} delay between the CP pulses (3 ms versus 1.5 ms) in both sequences. The *J*-modulation is slowed down further in $T_{2\rho}$-LASER compared to CP-LASER, especially for strongly coupled spin systems. Simulated spectra were in excellent agreement with the spectra measured in vivo (Figure 3).

Macromolecule resonances were also affected by the CP pulse train. For instance, the signal intensities for the 0.91 ppm and 1.7 ppm resonances were higher with CP-LASER and $T_{2\rho}$-LASER compared to LASER at similar echo times (Figure 2). However, no macromolecule signal was observed for any sequences at an echo time of 200 ms and longer.

Effect of CP pulse train on *J*-modulation

Under CP conditions, signal loss due to *J*-modulation was reduced much more significantly in strongly coupled spin systems than in weakly coupled spin systems as shown previously with simulations for alanine and taurine (26). This is illustrated in Figure 4 which shows simulations of total signal integral (without taking into account T_2 relaxation) as a function of T_E for glutamate (a strongly coupled spin system) and lactate (a weakly coupled spin system) for all three pulse sequences.

For glutamate, simulations show that *J*-modulation is substantially reduced with CP-LASER and $T_{2\rho}$-LASER compared to LASER, which is particularly apparent for $T_E < 150$ ms. For lactate, in contrast, *J*-modulation is not significantly slowed by the CP train for $T_E < 100$ ms, although it differs for each sequence at longer T_E. The signal integral of lactate with LASER follows a sinusoidal modulation as T_E increases, with the first minimum occurring at ~144 ms (i.e., 1/*J*) and the next maximum at ~288 Hz (i.e., 2/*J*) as expected for a weakly coupled ½ spin system. With the CP pulse train, the lactate integral is not refocused at multiples of 1/*J*.

This could also be observed in vivo (Figure 2): resonances of weakly coupled spins such as the methyl protons of lactate at 1.32 ppm and the NAA multiplet in the 2.49–2.67 ppm region showed noticeable *J*-modulation at much shorter T_E than resonances of strongly coupled spins such as glutamate, taurine and mIns, with CP-LASER and $T_{2\rho}$-LASER sequences.

Finally, simulations in Figure 4 also confirm that, while CP-LASER and $T_{2\rho}$-LASER behave very similarly at shorter T_E, significant differences in *J*-modulation become apparent at longer T_E, as can also be observed in experimental data (Figure 3).

Transverse relaxation times

The T_2 relaxation times of metabolites measured using all three pulse sequences are given in Table 1. T_2s of all measured metabolites were significantly longer with CP-LASER and $T_{2\rho}$-LASER compared to LASER. However, no significant difference was observed in measured T_2s between CP-LASER and $T_{2\rho}$-LASER sequences for any metabolite.

Singlet resonances showed a significant increase in T_2s with CP-LASER compared to LASER, ranging from 17% for tCho to 42% for NAA (Table 1). Similarly, the multiplet of NAA in the 2.47 to 2.67 ppm region showed a 6-19% increase in T_2 with CP-LASER and $T_{2\rho}$-LASER compared to LASER (Table 1). In comparison, the T_2 of water increased by 9% with CP-LASER and 16% with $T_{2\rho}$-LASER compared to LASER.

For strongly coupled systems, the increase in measured T_2s with CP-LASER and $T_{2\rho}$-LASER compared to LASER was much larger. For example, T_2 of glutamate increased from 70 ms with LASER to 296 ms with CP-LASER. The increase in T_2 with CP-LASER compared to LASER was about 2-fold for glutamine and taurine and about 4-fold for mIns and glutamate (Table 1). mIns exhibited the longest T_2 under CP condition (600 ms). This lengthening of T_2 is readily apparent when comparing the in vivo and simulated spectra of mIns and its resonance at 4.05 ppm (Figure 3B). Similarly, the prolonged T_2 was confirmed

directly for taurine using phantom data acquired with the three sequences (supplementary material).

The T_2 of tCho could not be determined under $T_{2\rho}$-LASER due to the strong cross-correlation between choline compounds and phosphorylethanolamine in the LCModel fit. In addition, the T_2 of lactate could not be determined reliably with CP-LASER and $T_{2\rho}$-LASER due to the lower signal integral of lactate with CP trains compared to LASER at most T_Es (Figure 4).

DISCUSSION

In this study, we report for the first time quantitative measurement of T_2 relaxation times for multiple *J*-coupled metabolites in the brain under CP conditions. While all metabolites had longer T_2 with CP-LASER and $T_{2\rho}$-LASER compared to LASER, the increase in T_2 was much more pronounced for strongly coupled spins (up to 4-fold longer) than for non-coupled (singlet) and weakly coupled spins. These findings provide new insights into the relaxation pathways in coupled spin systems and have significant implications for accurate quantification of MR spectra acquired at high field.

Comparison of T_2 measured with LASER, CP-LASER and $T_{2\rho}$-LASER

T_2s measured for non-coupled (singlet) spins including water were longer when measured with CP-LASER and $T_{2\rho}$–LASER sequences compared to LASER. However, the increase in T_2 was smaller for water than for other singlets such as the CH_3 group of NAA or the CH_3 and CH_2 groups of tCr. Similar observations have been previously reported for water, NAA and tCr in the human brain (5). For water which has a larger apparent diffusion coefficient (ADC) than metabolites in addition to rapidly exchanging protons, the increase in T_2 was smaller. This suggests that the diffusion component might not be efficiently refocused by the CP pulse trains (5). In contrast, NAA and tCr singlets have non-exchangeable protons and lower ADC than water, so that the CP pulse train suppresses the diffusion component efficiently, resulting in higher increase in T_2 under CP train.

Although the molecular structures of glutamate and glutamine are similar, these metabolites seem to experience different relaxation pathways under the CP pulse train. This is evidenced by the much higher increase in T_2s for glutamate (4-fold) compared to glutamine (2-fold) with CP-LASER and $T_{2\rho}$-LASER compared to LASER. This different gain may be explained by the higher diffusion coefficient for glutamine compared to glutamate as previously reported in the rat brain (27). With a high diffusion coefficient, CP pulse trains may not refocus the diffusion component of T_2 relaxation as efficiently (5).

Strongly coupled spins such as glutamate, taurine, and mIns exhibit a higher gain in T_2s under the CP pulse train compared to non-coupled spins although their diffusion coefficients are closer to that of singlet resonances. This suggests that the prolonged T_2 values cannot be solely attributed to suppression of the diffusion term since both non-coupled and *J*-coupled spins experience this relaxation mechanism. However, in strongly *J*-coupled systems, the additional increase in T_2 during a CP sequence can be attributed to refocusing of cross-correlation or interference effects (28) between different dipole-dipole interactions (29).

Finally, T_2s measured with CP-LASER and $T_{2\rho}$-LASER were very similar, although differences were observed in the *J*-modulation pattern at longer T_E. The similarity in T_2 values can be attributed to the relatively short τ_{cp} of 3 ms used in CP-LASER, resulting in minimal dephasing of magnetization during the interpulse interval (30). Longer τ_{cp} delays (> 3 ms) in CP-LASER would result in shorter $T_2^{\dagger}$ compared to $T_{2\rho}$. At very long τ_{cp}, $T_2^{\dagger}$ would approach that of T_{2f} as previously reported (16,30,31).

The transverse relaxation times are dependent on magnetic field strength and the properties of the pulse sequence utilized (i.e., RF pulse patterns and timings) such that T_2 values and T_2 gains observed in this study at 9.4 T might not hold true for other sequences and other timings. For example, the T_2s of singlets (e.g., NAA, tCr and tCho) measured with LASER in the present study were consistent with a previous rat study using a similar pulse sequence (7) but were longer (between 30% to 250% longer) than those reported with PRESS or SPECIAL sequences (32,33) at 9.4 T. Additionally, T_2 values measured with LASER but not with CP-LASER and $T_{2\rho}$-LASER can be affected by the chemical shift displacement errors. In the present study, the largest chemical shift displacement error of 7% would be observed for lactate due to the large bandwidth of the AFP pulses. However, for the strongly coupled metabolites such as glutamate, glutamine, taurine and mIns the chemical shift displacement error would be smaller (4%, 4%, 0.4%, and 2%, respectively).

Comparison of effect of CP train in human brain and rat brain

The increase in T_2 under CP conditions (i.e., $T_2^{\dagger}/T_{2f}$) is dependent on B_0, as reported previously (5), because the dynamic dephasing (i.e., diffusion and exchange) component of transverse relaxation increases with B_0. The T_2s measured for non-coupled spins (NAA, tCr and tCho) with CP-PRESS (12) in human brain at 1.5 T were only slightly longer compared to values obtained with the PRESS sequence (34). At higher fields, however, the increase in T_2 with CP-LASER compared to PRESS was larger. The T_2 of NAA singlet in human brain was 1.7 times longer at 4 T and 2.2 longer at 7 T with CP-LASER compared to PRESS (5), and 1.5 times longer at 4 T with CP-LASER (5) compared to LASER (6).

In the present study in rat brain, the increase in the T_2 of NAA singlet at 9.4 T with CP-LASER compared to LASER was only a factor 1.4, which was lower than the increase observed in human brain at 4 T. Therefore the increase in T_2 with CP-LASER compared to LASER appears smaller in rat brain than in human brain at a given B_0. This is likely due to smaller microsusceptibility effects in rat brain, resulting in smaller B_0 dephasing effects (35,36). A similar dependence of T_2 on microsusceptibility and magnetic field can be expected for *J*-coupled spins.

Effect of RF pulse modulation on T_2 relaxation times

The increase in T_2 during CP pulse trains depends both on the amplitude and frequency-modulation functions of the AFP pulses used (37). It was experimentally demonstrated on phantom using ^{1}H MRS that water exhibits longer $T_{2\rho}$ relaxation times under HS4 AFP pulses compared to HS1 AFP pulses with similar pulse duration and B_1 field (31,38,39). Similar findings were recently reported for the CH_3 group of NAA and tCr in the human brain in vivo at 4 T (39). This dependence of T_2 on modulation functions is the result of

spins experiencing different dipole-dipole and/or chemical exchange interactions during the different type of pulses (37) and forms the basis of a new MRI contrast (31). In the present study, HS1 pulses were used for both CP train as well as in LASER. Even larger increases in T_2 can be expected if HS4 pulses are used.

Absolute quantification

Sequences such as LASER and semi-LASER are advantageous at high field because AFP pulses can be lengthened while retaining a large refocusing bandwidth, which minimizes chemical-shift displacement artifacts. Even though LASER and semi-LASER have longer T_E than STEAM, the effect of this longer T_E is mitigated by reduced *J*-modulation and increased T_2. In fact, it has been shown that LASER and semi-LASER spectra can be very similar in appearance to short echo-time STEAM spectra while providing 2-fold higher signal-to-noise.

At high magnetic fields, the minimum achievable T_E generally becomes longer due to increased RF power requirements, which leads to longer RF pulses. At the same time, T_2s relaxation times become shorter at high field (36). Therefore, while often neglected at lower field, signal loss due to T_2 relaxation may not be negligible at high field, especially in human brain.

Correction for T_2 relaxation requires measurement of the T_2s of metabolites. Our results show that the common practice of measuring T_2 by inserting free precession delays does not provide accurate T_2 values for CP-like sequences. For this type of sequences such as LASER and semi-LASER, T_2s must be measured while keeping τ_{cp} constant to take into account the effect of successive 180° pulses. The potential effect of successive 180° pulses on T_2 is important to keep in mind when performing absolute quantification.

CONCLUSION

This study reports for the first time quantitative measurements of T_2s for multiple *J*-coupled metabolites. It is shown that the use of a CP pulse train results in a much larger increase in T_2 for strongly coupled spins (up to 4-fold) than for non-coupled and weakly coupled spins. Therefore, pulse sequences with a succession of 180° pulses such as LASER are particularly advantageous for retaining signals from strongly coupled spins at relatively short T_E. If correcting for T_2 relaxation is needed in LASER and semi-LASER, care must be taken to use T_2 values measured under CP-like conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Chris Nelson, Manda Vollmers and Emily Colonna for expert technical assistance, Dr. Shalom Michaeli for helpful discussions and Dr. Jamie Walls for reading the manuscript. This work was supported by funding from NIH grants P41 EB015894 and P30 NS076408 and the W.M. KECK Foundation.

REFERENCES

1. Garwood M, DelaBarre L. The return of the frequency sweep: designing adiabatic pulses for contemporary NMR. J Magn Reson. 2001; 153(2):155–177. [PubMed: 11740891]
2. Scheenen TWJ, Klomp DWJ, Wijnen JP, Heerschap A. Short echo time 1H-MRSI of the human brain at 3T with minimal chemical shift displacement errors using adiabatic refocusing pulses. Magn Reson Med. 2008; 59(1):1–6. [PubMed: 17969076]
3. Marjanska, Mg; Auerbach, EJ.; Valabregue, R. Van de Moortele P-F, Adriany G, Garwood M. Localized 1H NMR spectroscopy in different regions of human brain in vivo at 7T: T2 relaxation times and concentrations of cerebral metabolites. NMR Biomed. 2012; 25(2):332–339. [PubMed: 21796710]
4. Oz G, Tkac I. Short-echo, single-shot, full-intensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: Validation in the cerebellum and brainstem. Magn Reson Med. 2011; 65(4):901–910. [PubMed: 21413056]
5. Michaeli S, Garwood M, Zhu XH, DelaBarre L, Andersen P, Adriany G, Merkle H, Ugurbil K, Chen W. Proton T_2 relaxation study of water, N-acetylaspartate, and creatine in human brain using Hahn and Carr-Purcell spin echoes at 4T and 7T. Magn Reson Med. 2002; 47(4):629–633. [PubMed: 11948722]
6. Deelchand DK, Henry P-G, Ugurbil K, Marjanska M. Measurement of transverse relaxation times of J-coupled metabolites in the human visual cortex at 4 T. Magn Reson Med. 2012; 67(4):891–897. [PubMed: 21748799]
7. de Graaf R, Brown P, McIntyre S, Nixon T, Behar K, Rothman D. High magnetic field water and metabolite proton T_1 and T_2 relaxation in rat brain in vivo. Magn Reson Med. 2006; 56(2):386–394. [PubMed: 16767752]
8. Marjanska M, Curran GL, Wengenack TM, Henry PG, Bliss RL, Poduslo JF, Jack CR Jr. Ugurbil K, Garwood M. Monitoring disease progression in transgenic mouse models of Alzheimer's disease with proton magnetic resonance spectroscopy. Proc Natl Acad Sci U S A. 2005; 102(33):11906–11910. [PubMed: 16091461]
9. Mangia S, Liimatainen T, Garwood M, Tkac I, Henry P-G, Deelchand D, Michaeli S. Frequency offset dependence of adiabatic rotating frame relaxation rate constants: relevance to MRS investigations of metabolite dynamics in vivo. NMR Biomed. 2011; 24(7):807–814. [PubMed: 21264976]
10. Carr HY, Purcell EM. Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. Phys Rev. 1954; 94(3):630–638.
11. Allerhand A. Analysis of Carr---Purcell Spin-Echo NMR Experiments on Multiple-Spin Systems. I. The Effect of Homonuclear Coupling. J Chem Phys. 1966; 44(1):1–9.
12. Soher BJ, Pattany PM, Matson GB, Maudsley AA. Observation of coupled ^{1}H metabolite resonances at long T_E. Magn Reson Med. 2005; 53(6):1283–1287. [PubMed: 15906305]
13. Hennig J, Thiel T, Speck O. Improved sensitivity to overlapping multiplet signals in in vivo proton spectroscopy using a multiecho volume selective (CPRESS) experiment. Magn Reson Med. 1997; 37(6):816–820. [PubMed: 9178230]
14. Hancu I, Gillen R, Cowan J, Zimmerman EA. Improved myo-inositol detection through Carr-Purcell PRESS: A tool for more sensitive mild cognitive impairment diagnosis. Magn Reson Med. 2011; 65(6):1515–1521. [PubMed: 21590800]
15. Allerhand A, Thiele E. Analysis of Carr---Purcell Spin-Echo NMR Experiments on Multiple-Spin Systems. II. The Effect of Chemical Exchange. J Chem Phys. 1966; 45(3):902–916.
16. Bartha R, Michaeli S, Merkle H, Adriany G, Andersen P, Chen W, Ugurbil K, Garwood M. In vivo ^1H_2O T^+_2 measurement in the human occipital lobe at 4T and 7T by Carr- Purcell MRI: detection of microscopic susceptibility contrast. Magn Reson Med. 2002; 47(4):742–750. [PubMed: 11948736]
17. Hennig J, Nauerth A, Friedburg H. RARE imaging: A fast imaging method for clinical MR. Magn Reson Med. 1986; 3(6):823–833. [PubMed: 3821461]
18. Gruetter R, Tkac I. Field mapping without reference scan using asymmetric echo-planar techniques. Magn Reson Med. 2000; 43(2):319–323. [PubMed: 10680699]

19. Silver MS, Joseph RI, Hoult DI. Highly selective π/2 and π pulse generation. J Magn Reson. 1984; 59(2):347–351.
20. Levitt MH, Freeman R, Frenkiel T. Supercycles for broadband heteronuclear decoupling. J Magn Reson. 1982; 50(1):157–160.
21. Tkac I, Starcuk Z, Choi IY, Gruetter R. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. Magn Reson Med. 1999; 41(4):649–656. [PubMed: 10332839]
22. Pfeuffer J, Tkac I, Provencher SW, Gruetter R. Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time ^{1}H NMR spectra of the rat brain. J Magn Reson. 1999; 141(1):104–120. [PubMed: 10527748]
23. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med. 1993; 30(6):672–679. [PubMed: 8139448]
24. Henry PG, Marjanska M, Walls JD, Valette J, Gruetter R, Ugurbil K. Proton-observed carbon-edited NMR spectroscopy in strongly coupled second-order spin systems. Magn Reson Med. 2006; 55(2):250–257. [PubMed: 16402370]
25. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed. 2000; 13(3):129–153. [PubMed: 10861994]
26. Mayer D, Dreher W, Leibfritz D, Spielman DM. RF refocused echoes of J-coupled spin systems: Effects on RARE-based spectroscopic imaging. Magn Reson Med. 2007; 57(5):967–971. [PubMed: 17457878]
27. Pfeuffer J, Tkac I, Gruetter R. Extracellular-intracellular distribution of glucose and lactate in the rat brain assessed noninvasively by diffusion-weighted 1H nuclear magnetic resonance spectroscopy in vivo. J Cereb Blood Flow Metab. 2000; 20(4):736–746. [PubMed: 10779018]
28. Blicharski JS, Kruk D. NMR relaxation spectroscopy: Interference effects. Appl Magn Reson. 1999; 17(2-3):367–374.
29. Pervushin K, Riek R, Wider G, Wuthrich K. Attenuated T2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. Proc Natl Acad Sci U S A. 1997; 94(23):12366–12371. [PubMed: 9356455]
30. Santyr GE, Henkelman RM, Bronskill MJ. Variation in measured transverse relaxation in tissue resulting from spin locking with the CPMG sequence. J Magn Reson. 1988; 79:28–44.
31. Michaeli S, Gröhn H, Gröhn O, Sorce DJ, Kauppinen R Jr. Ugurbil K, Garwood M. Exchange-influenced T_2rho contrast in human brain images measured with adiabatic radio frequency pulses. Magn Reson Med. 2005; 53(4):823–829. CSS. [PubMed: 15799068]
32. Xin L, Gambarota G, Mlynarik V, Gruetter R. Proton T_2 relaxation time of J-coupled cerebral metabolites in rat brain at 9.4 T. NMR Biomed. 2008; 21(4):396–401. [PubMed: 17907262]
33. Lei H, Zhang Y, Zhu XH, Chen W. Changes in the proton T_2 relaxation times of cerebral water and metabolites during forebrain ischemia in rat at 9.4 T. Magn Reson Med. 2003; 49(6):979–984. [PubMed: 12768573]
34. Kreis R. Quantitative localized ^{1}H MR spectroscopy for clinical use. Prog Nucl Magn Reson Spectrosc. 1997; 31(2-3):155–195.
35. Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. Proc Natl Acad Sci U S A. 2000; 97(10):5621–5626. DO -5610.1073/pnas.090504197. [PubMed: 10792049]
36. Deelchand DK, Moortele P-FVd, Adriany G, Iltis I, Andersen P, Strupp JP, Thomas Vaughan J, Ugurbil K, Henry P-G. In vivo ^{1}H NMR spectroscopy of the human brain at 9.4 T: Initial results. J Magn Reson. 2010; 206(1):74–80. [PubMed: 20598925]
37. Michaeli S, Sorce DJ, Idiyatullin D, Ugurbil K, Garwood M. Transverse relaxation in the rotating frame induced by chemical exchange. J Magn Reson. 2004; 169(2):293–299. [PubMed: 15261625]
38. Mangia S, Liimatainen T, Garwood M, Michaeli S. Rotating frame relaxation during adiabatic pulses vs. conventional spin lock: simulations and experimental results at 4 T. Magn Reson Imaging. 2009; 27(8):1074–1087. [PubMed: 19559559]
39. Mangia S, Garwood M, Tkac I, Ugurbil K, Michaeli S. Probing dynamics of human brain metabolites with 1H MRS by T1rho and T2rho adiabatic relaxations. Proc Intl Soc Mag Reson Med. 2008; (16):693.

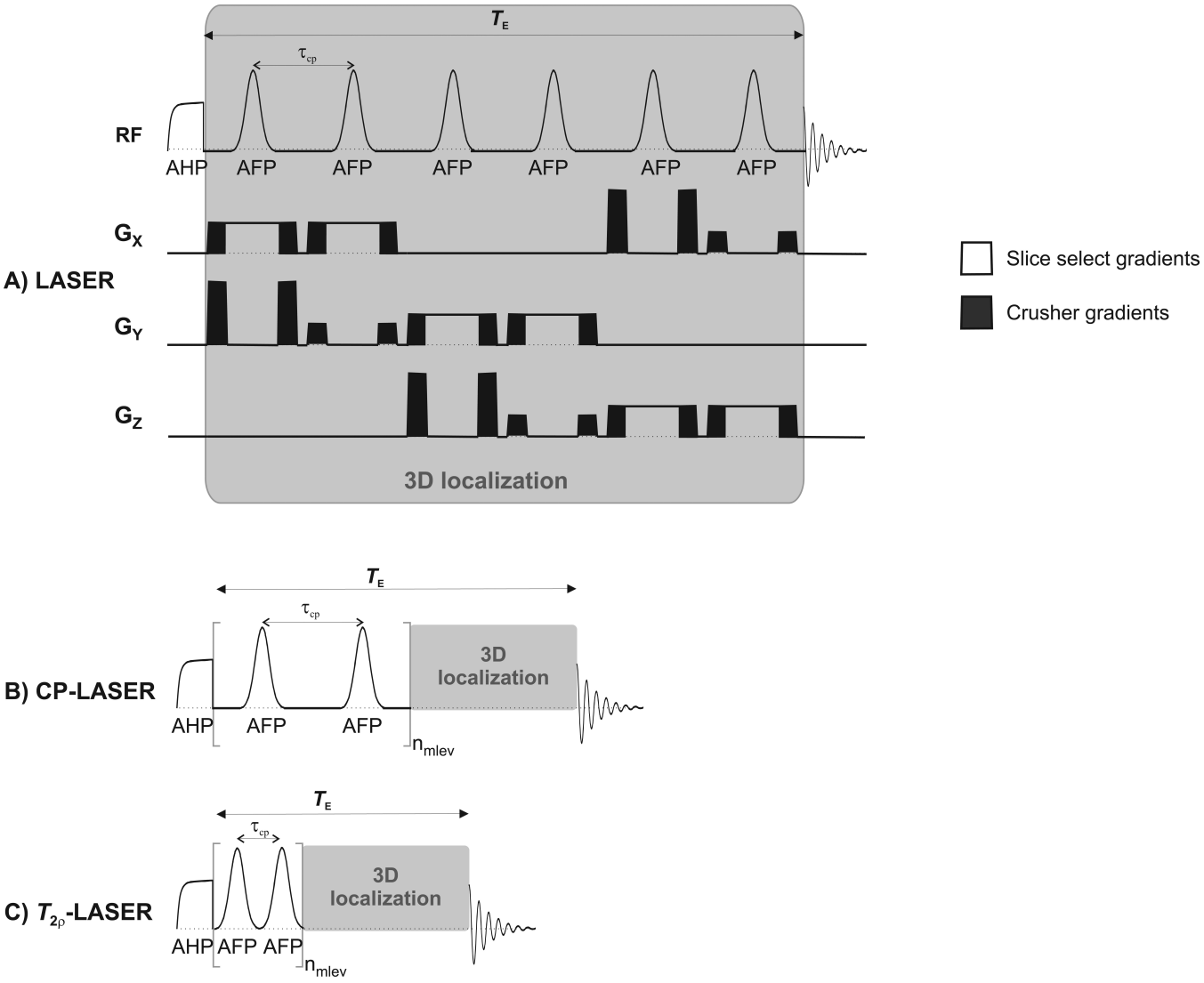

Figure 1.

Diagrams of A) LASER, B) CP-LASER and C) $T_{2\rho}$-LASER sequences used for MRS acquisition. In LASER, the AFP pulses were applied in the presence of gradients for 3D localization (represented by the gray box). In CP- and $T_{2\rho}$- LASER sequences, a CP train of AFP pulses (identical adiabatic pulses as utilized in LASER) was inserted prior to the LASER localization and was phase cycled according to the MLEV scheme. The τ_{cp} delay was 3 ms in CP-LASER and 1.5 ms in $T_{2\rho}$-LASER (equivalent to the duration of one AFP pulse). No spoiler gradients were applied during the CP segment.

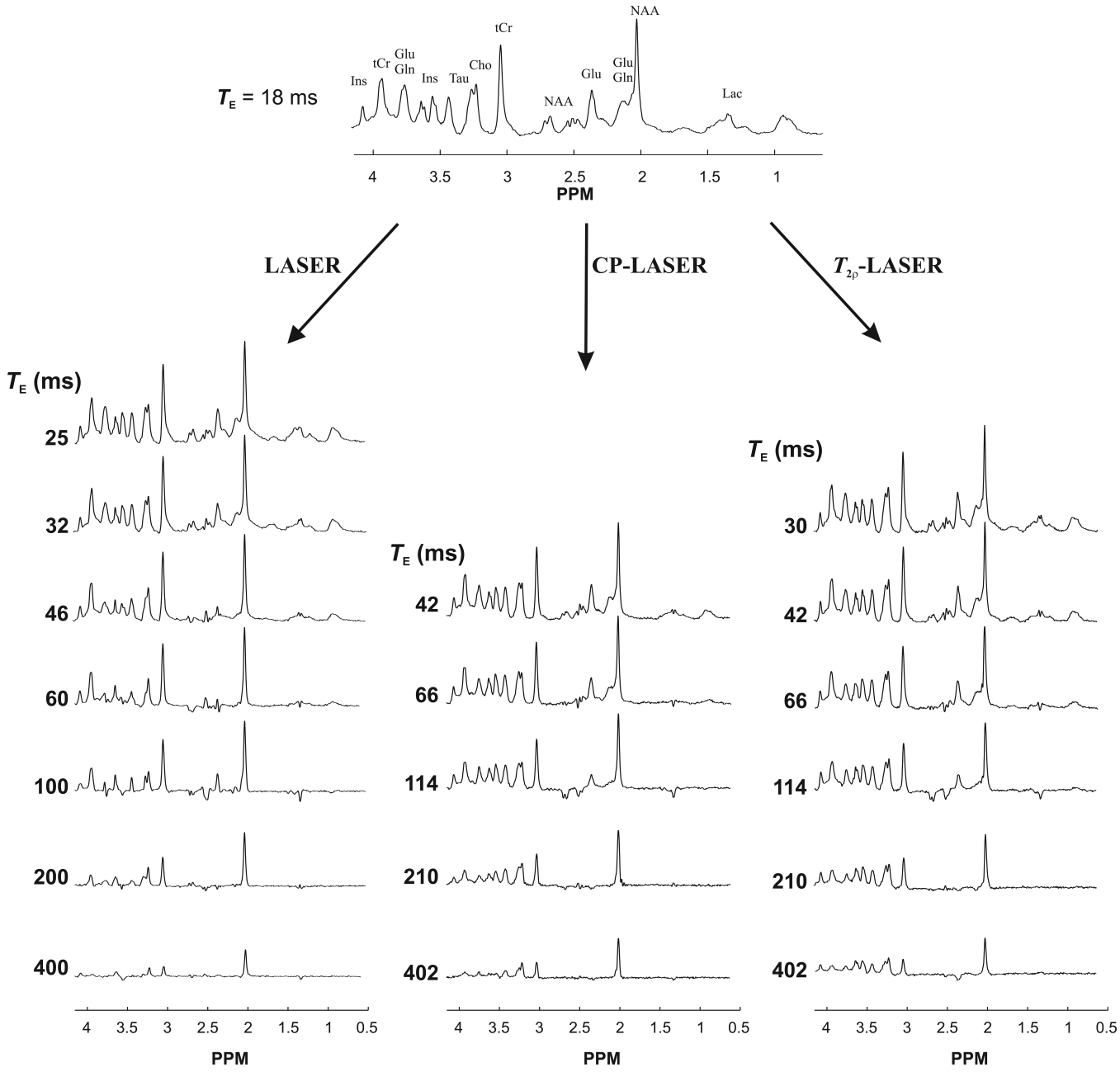

Figure 2.

In vivo ^{1}H NMR spectra measured with LASER, CP-LASER and $T_{2\rho}$-LASER sequences from the same animal with different T_Es (T_R = 4 s, 128 averages were acquired for all spectra except for T_E = 400 ms in LASER where 192 averages were acquired). The $T_{2\rho}$-LASER spectrum at 402 ms was only acquired in this animal to show that even at such long T_E several resonances in the 3 to 4.2 ppm range could be still observed compared to LASER and CP-LASER spectra.

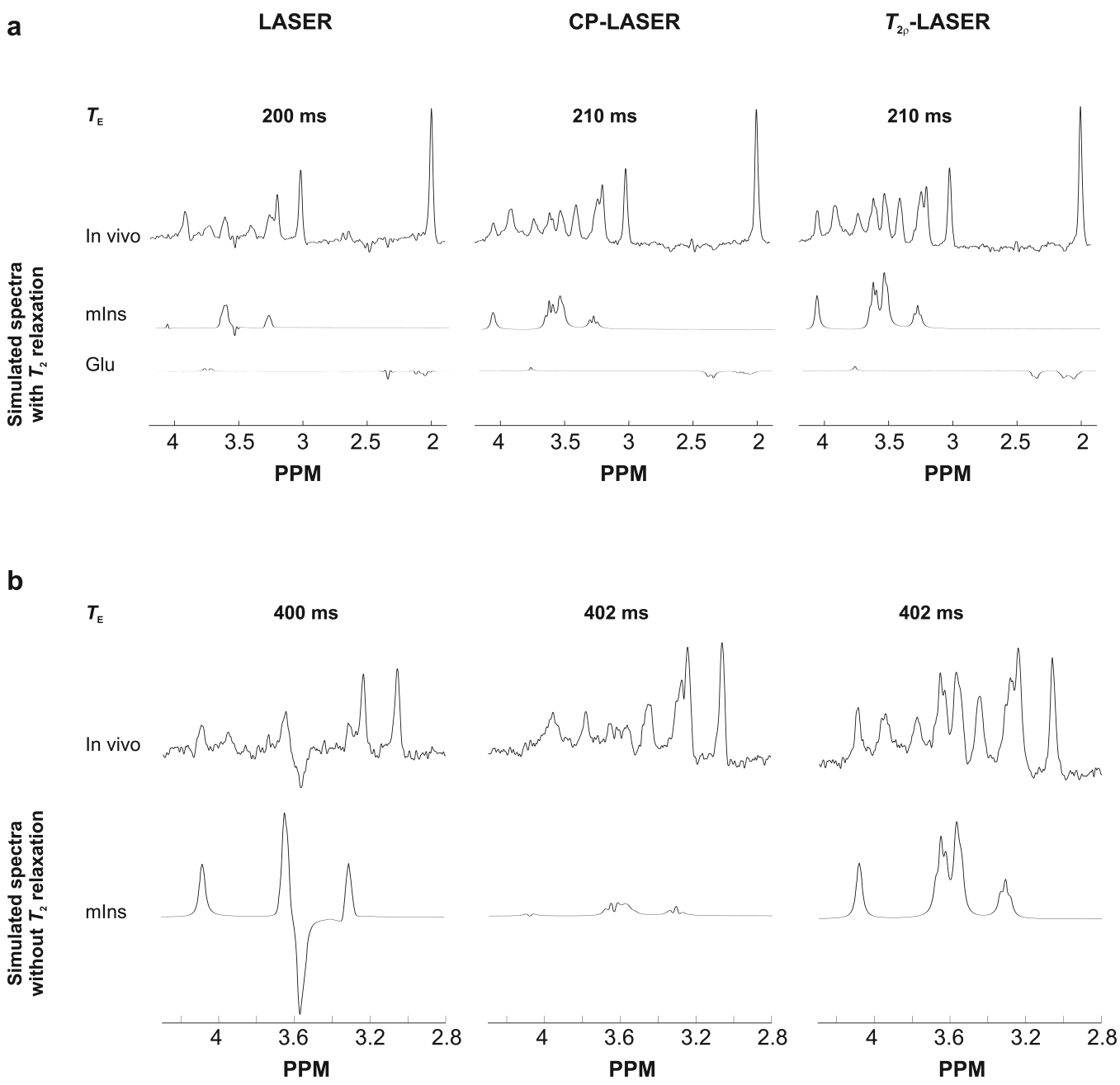

Figure 3.

Comparison of LASER, CP-LASER and $T_{2\rho}$-LASER spectra (128 averages, a 1.5 Hz Gaussian line broadening was applied prior to FT) measured at (a) T_E = 200 ms and 210 ms and (b) T_E = 400 ms and 402 ms from the same animal. In (a), the simulated spectra are scaled by T_2 relaxation (amplitude matched to in vivo spectra). The observed differences in intensities between CP-LASER and $T_{2\rho}$-LASER sequences are consistent with the density-matrix simulated NMR spectra. In (b), the simulated mIns spectra are shown with the same scaling adjusted so the intensity of mIns in $T_{2\rho}$-LASER simulated spectrum matches the intensity of mIns in the $T_{2\rho}$-LASER in vivo spectrum. Therefore the simulated spectra represent the relative signals intensity of mIns that would be expected if T_2 was the same

with LASER, CP-LASER and $T_{2\rho}$-LASER. With the same T_2 relaxation, the peak at 4.05 ppm would have almost identical amplitude with LASER and $T_{2\rho}$-LASER. However, it is apparent that in vivo this peak is much smaller in LASER spectrum compared to $T_{2\rho}$-LASER spectrum providing direct visual evidence that the T_2 of mIns is longer with T_2-LASER compared with LASER.

The simulated spectra are shown line-broadened to match in vivo spectra (8 Hz exponential line-broadening). Glu = glutamate, mIns = *myo*-inositol.

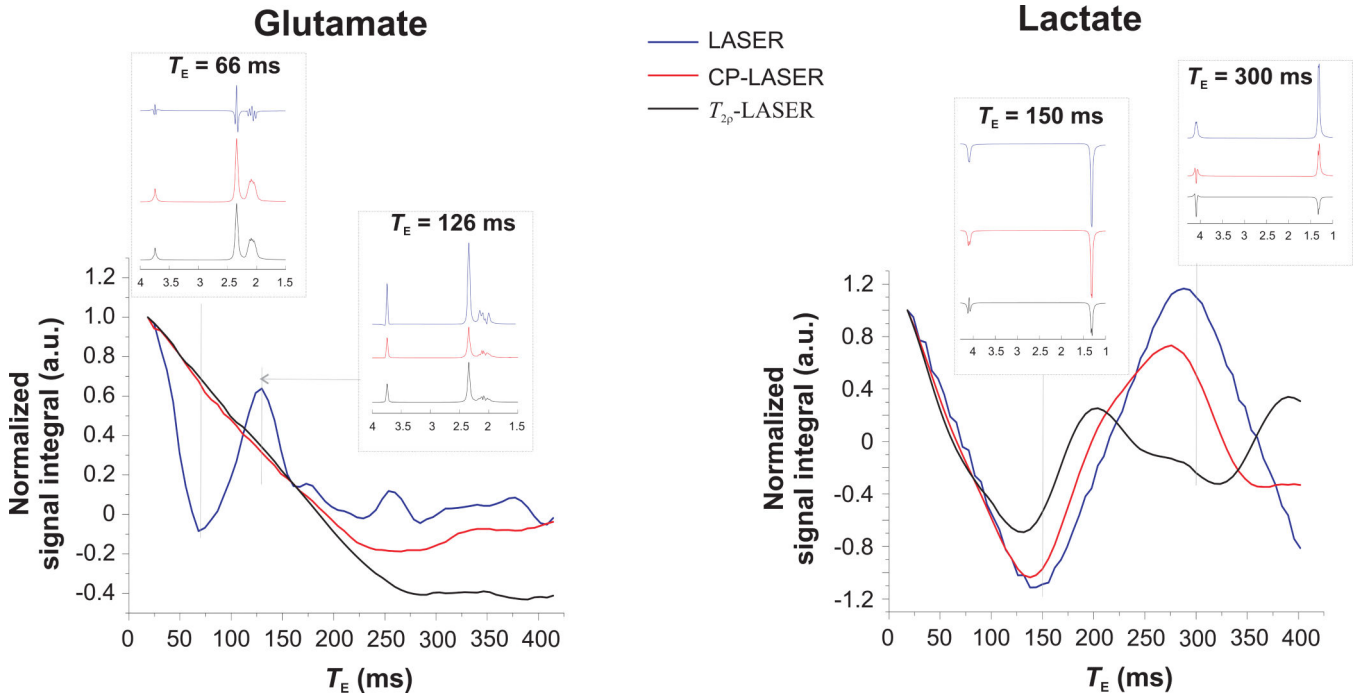

Figure 4.

Effect of CP pulse trains on the *J*-evolution of glutamate (strongly coupled system) and lactate (weakly coupled system). Signal integral (without taking into account T_2 relaxation) which corresponds to the amplitude of the first point of the simulated FID normalized to 1 at T_E of 18 ms is shown as a function of T_E for LASER (blue line), CP-LASER (black line) and $T_{2\rho}$-LASER (red line) sequences at 9.4 T. Inserts show the spectral pattern of glutamate at T_E = 66 and 126 ms, and lactate at T_E = 150 and 300 ms for the three sequences. Although the signal integral of glutamate with LASER is almost zero at T_E = 66 ms, there is sufficient signal intensity for precise LCModel fitting. At T_E = 126 ms, the signal intensity (without T_2 relaxation) of glutamate with LASER is 2.5 times higher than that with $T_{2\rho}$-LASER. It is possible that for certain T_Es and certain metabolites, LASER sequence might have more signal than CP-LASER or $T_{2\rho}$-LASER.

The simulated spectra are shown line-broadened (10 Hz exponential line-broadening).

Table 1

T_2 relaxation times (mean ± SD) measured with LASER, CP-LASER and $T_{2\rho}$-LASER sequences and T_2 ratios for CP-LASER and LASER and $T_{2\rho}$-LASER and LASER obtained in the rat brain (n = 6) in vivo at 9.4 T. T_2s of all measured metabolites were significantly longer ($P < 0.05$) with CP pulse sequences compared to LASER. T_2 of water was also significantly longer with CP-LASER ($P < 0.01$) and $T_{2\rho}$-LASER ($P < 0.001$) compared to LASER. Between CP-LASER and $T_{2\rho}$-LASER sequences, the T_2s of metabolites were not significantly different ($P > 0.05$) except in the case of water ($P = 0.04$). The high SDs observed for tCho and mIns were due to their T_2 being longer than the maximum T_E used in this study. The goodness of fit, R^2 was > 0.9 for all metabolites except for the multiplet of NAA which was 0.88 with LASER.

Spin systems	Compound	Group	LASER T_{2f} (ms)	CP-LASER $T_2^{\dagger}$ (ms)	$T_{2\rho}$-LASER $T_{2\rho}$ (ms)	$T_2^{\dagger}/T_{2f}$	$T_{2\rho}/T_{2f}$
Non-coupled and weakly coupled	Water	H_2O	44 ± 2	48 ± 2	50 ± 2	1.09 ± 0.07	1.16 ± 0.07
	NAA	$^2CH_3$	321 ± 30	456 ± 26	449 ± 32	1.42 ± 0.16	1.40 ± 0.17
	NAA	$^3CH_2$	157 ± 41	187 ± 16	167 ± 25	1.19 ± 0.33	1.06 ± 0.32
	tCr	$N(CH_3)$	170 ± 12	224 ± 7	231 ± 16	1.32 ± 0.10	1.36 ± 0.13
	tCr	$^2CH_2$	146 ± 16	175 ± 9	183 ± 20	1.20 ± 0.15	1.25 ± 0.20
	tCho	entire molecule	445 ± 67	523 ± 68	ND	1.17 ± 0.23	-
Strongly coupled	Glu	entire molecule	70 ± 14	296 ± 44	270 ± 21	4.20 ± 1.02	3.84 ± 0.80
	Gln	entire molecule	56 ± 12	116 ± 7	126 ± 19	2.08 ± 0.45	2.26 ± 0.58
	mIns	entire molecule	161 ± 26	584 ± 94	600 ± 90	3.62 ± 0.82	3.72 ± 0.82
	taurine	entire molecule	162 ± 28	355 ± 17	343 ± 27	2.19 ± 0.39	2.11 ± 0.40