

In vivo ^1H MR spectroscopy with J -refocusing

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Purpose: The goal of this study was to propose a novel localized proton MR spectroscopy (MRS) sequence that reduces signal loss due to J -modulation in the rat brain in vivo.

Methods: Sprague-Dawley rats were studied at 9.4 T. A semi-LASER sequence with evenly distributed echo-time (T_E) was used, and a 90° J -refocusing pulse was inserted at $T_E/2$. Proton spectra were acquired at two T_E s (30 and 68 ms), with and without the J -refocused pulse. Data were processed in MATLAB and quantified with LCModel.

Results: The J -refocused spectrum acquired at $T_E = 30$ ms did not show any signal losses due to J -modulation and had comparable spectral pattern to the one acquired with semi-LASER using the minimum achievable T_E . Higher signal amplitudes for glutamine, γ -aminobutyric acid and glutathione led to more reliable quantification precision for these metabolites. The refocused signal intensities at $T_E = 68$ ms were also unaffected by J -modulation but were smaller than the signals at $T_E = 30$ ms mainly due to transverse T_2 relaxation of metabolites.

Conclusion: The proposed localized MRS sequence will be beneficial in both animal and human MRS studies when using ultra-short T_E is not possible while also providing more reliable quantification precision for J -coupled metabolites.

KEYWORDS

9.4 T, brain, GABA, glutamine, glutathione, J -coupled metabolites, semi-LASER

1 | INTRODUCTION

Proton MR spectroscopy (MRS) enables non-invasive measurement of many metabolites in the brain. Certain metabolites, such as creatine and phosphocreatine, have only singlet resonances while other metabolites, such as N -acetylaspartate (NAA), phosphorylcholine (PCho), and glycerophosphorylcholine (GPC), consist of both singlet and multiplet peaks. In addition, a number of metabolites, such as glutamate (Glu), glutamine (Gln), γ -aminobutyric acid (GABA), and glutathione (GSH), have only J -coupled resonances.^{1,2} At ultra-short echo-times (T_E s), these J -coupled resonances are represented by in-phase multiplets that are relatively easy to quantify (based on their appropriate concentration levels) with prior

knowledge in the fit analysis. As T_E increases, however, these J -coupled metabolites undergo J -modulation, and their spectral patterns change as a function of T_E .³ Based on the J -coupling constants and chemical shift differences between J -coupled partners, the spectral patterns might become completely dephased at a specific T_E , as in the case of Glu at 9.4 T and $T_E = 45$ ms. In such instances, quantification of those metabolites might be difficult or impossible. To avoid these T_E -related issues, several studies⁴⁻⁶ measuring T_2 relaxation time constants of metabolites have used density-matrix simulations to determine the appropriate T_E values to use for quantifying either specific metabolites or the entire neurochemical profile.

To minimize J -modulation of the spectrum for coupled spin systems, the Carr-Purcell-Meiboom-Gill (CPMG) sequence,

which consists of multiple 180° pulses, is most commonly used.⁷ We have successfully shown that J -evolution can be reduced in the rat brain in vivo at 9.4 T using CP-LASER and $T_{2\rho}$ -LASER sequences.⁸ However, owing to the multiple 180° pulses required for CPMG, this technique cannot be easily translated to human studies due to specific absorption rate (SAR) deposition issues at fields of 3 T and above.

Another interesting technique to suppress J -modulation, which was proposed in the late 1980s, is to apply a 90° pulse with an appropriate phase at the midpoint of a double spin-echo sequence.^{9,10} This method seems promising for human studies due to the negligible SAR requirement of using only a single 90° pulse compared with the multiple 180° pulses required for conventional CPMG techniques. Several groups have shown the efficiency of suppressing J -modulation on phantom and tissue extracts using proton MRS,^{11–14} and one group applied this technique in vivo with a focus on glutamate

detection using a complex pulse sequence that required four shots for 3D localization.¹⁵ However, up to now, no study has successfully demonstrated this technique to work for a range of J -coupled metabolites that are present in the brain in vivo. Therefore, the aim of this study is to propose a novel sequence that enables the acquisition of localized proton MRS data with J -refocusing for coupled metabolites in the rat brain in vivo. The sequence, which is given in Figure 1A, is based on the semi-LASER^{16,17} with an evenly distributed T_E .

2 | METHODS

2.1 | Localized J -refocused sequence

Several high-resolution NMR and phantom studies have previously demonstrated refocusing of J -modulation for weakly

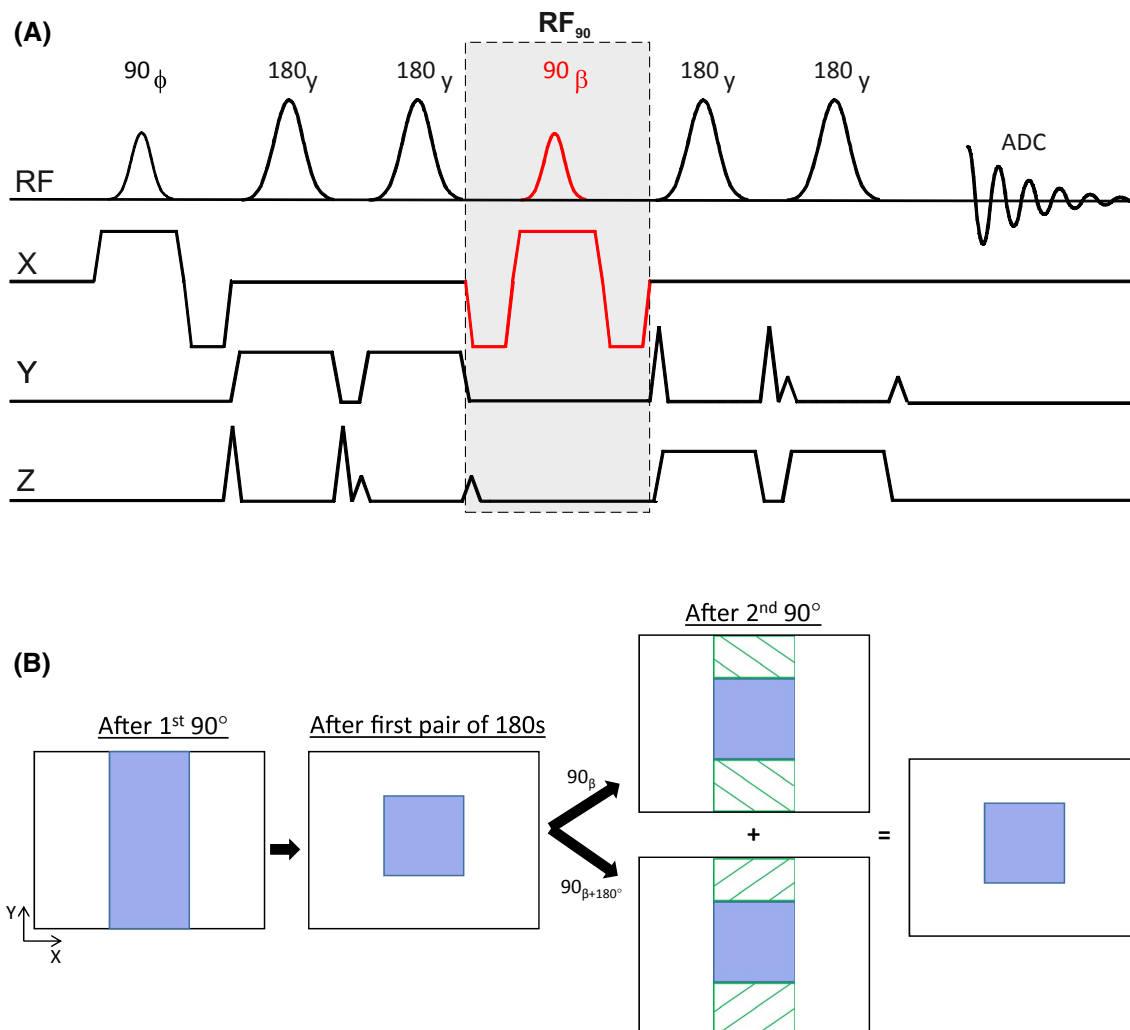


FIGURE 1 A, Semi-LASER sequence with an additional slice-selective 90° pulse (RF₉₀ inside gray box) to suppress J -modulation of coupled metabolites. The phase of the RF₉₀ pulse is $+90^\circ$ relative to the first excitation pulse, e.g., if $\phi = 0^\circ$ or 90° then $\beta = 90^\circ$ or 180° , respectively. The sequence in A becomes a conventional semi-LASER by removing the RF₉₀ pulse and the dephasing refocusing gradients about the RF₉₀ pulse. B, Schematic depicting VOI selection in the transverse XY plane. After the first pair of adiabatic full passage (AFP) pulses, a bar selection (blue box) is achieved along Z-direction. However, applying the RF₉₀ pulse refocuses signal outside the VOI along the X-direction (green striped box). The signals from a two-step phase cycle of the RF₉₀ phase, β and $\beta+180^\circ$, are added to eliminate signal outside the VOI. ADC, analog-to-digital converter

and/or strongly coupled spin systems.⁹⁻¹³ A double spin-echo sequence has been commonly used whereby a 90° pulse (denoted herein as RF₉₀) is inserted at the time where the first echo occurs, i.e., at T_E/2, and the phase of the RF₉₀ pulse was set to be +90° relative to that of the first excitation pulse. In this work, the semi-LASER^{16,17} was considered as the most suitable pulse sequence to incorporate the RF₉₀ pulse, since it consists of an excitation pulse followed by two pairs of refocusing adiabatic full passage (AFP) pulses.

A 3D localized semi-LASER sequence was implemented by inserting a RF₉₀ pulse at time T_E/2 (Figure 1A). The T_E was evenly distributed such that the inter-pulse delay between the AFP pulses was identical. The RF₉₀ was slice-selective and applied along the X-direction as was the first slice-selective excitation pulse. Dephasing and rephasing gradients were applied before and after the RF₉₀ pulse to make sure that the phase at the center of this pulse was zero for all spins in the selected slab.¹⁸ In this configuration, unwanted signal from outside the volume of interest (VOI) was refocused along the X-direction as shown in the schematic in Figure 1B. To eliminate such outside excitation, a simple two-step phase cycle on the RF₉₀ pulse was used such that the signals with RF₉₀ pulse phases of β and β+180° were added. Another option is to use a global non slice-selective RF₉₀ pulse. In this case, T_E would

were simulated with and without the refocusing RF₉₀ pulse using the actual RF pulse durations (without gradients) and patterns as used for the in vivo studies described in the next section.

2.2 | Theoretical understanding of J-refocusing

While J-refocusing is perfect for a weakly coupled IS spin system,⁹ J-evolution in strongly and weakly coupled arbitrary spin systems can still be refocused to a high degree¹² as long as $\pi J_{\max} \frac{T_E}{2} \ll 1$, where J_{max} is the maximum spin-spin coupling within a molecule. To see this, consider the spin evolution in a weakly coupled spin system under the J-refocused sequence, which can be effectively described by a RF_{90Y}-T_E/2-RF_{90X}-T_E/2 pulse sequence where chemical shift evolution has been removed by the AFP pulse pairs leaving evolution under the spin-spin Hamiltonian in the weak-coupling limit, $H = 2\pi \sum_{j < k} J_{jk} I_{Z,j} I_{Z,k}$, during the T_E/2 periods.

In this case, the spin evolution under the J-refocused sequence can be described by the following set of transformations:

$$\begin{aligned} \sum_j I_{Z,j} &\xrightarrow{90^\circ Y} \sum_j I_{X,j} \xrightarrow{\frac{T_E}{2}} \sum_j I_{X,j} f_j \left(\frac{T_E}{2} \right) + 2 \sum_{k \neq j} g_{jk} \left(\frac{T_E}{2} \right) I_{Y,j} I_{Z,k} \\ &+ O \left[\left(\frac{\pi J T_E}{2} \right)^2 \right] \xrightarrow{90^\circ X} \sum_j I_{X,j} f_j \left(\frac{T_E}{2} \right) - 2 \sum_{k \neq j} g_{kj} \left(\frac{T_E}{2} \right) I_{Y,j} I_{Z,k} \\ &+ O \left[\left(\frac{\pi J T_E}{2} \right)^2 \right] \xrightarrow{\frac{T_E}{2}} \sum_j I_{X,j} \left(\left(f_j \left(\frac{T_E}{2} \right) \right)^2 + \sum_{k \neq j} g_{kj} \left(\frac{T_E}{2} \right) g_{jk} \left(\frac{T_E}{2} \right) \right) \\ &\quad + 2 \sum_{k \neq j} I_{Y,j} I_{Z,k} f_j \left(\frac{T_E}{2} \right) \left(g_{jk} \left(\frac{T_E}{2} \right) - g_{kj} \left(\frac{T_E}{2} \right) \right) \\ &+ O \left[\left(\frac{\pi J T_E}{2} \right)^2 \right] \equiv \sum_j \text{Signal}_{j,\text{in-phase}}^{\text{Refocused}} (T_E) I_{X,j} + 2 \sum_{k \neq j} \text{Signal}_{jk,\text{anti-phase}}^{\text{Refocused}} (T_E) I_{Y,j} I_{Z,k} + O \left[\left(\frac{\pi J T_E}{2} \right)^2 \right] \end{aligned} \quad (1)$$

be further shortened on the order of 0.5 to 1 ms on animal scanners and approximately 2 to 3 ms on human scanners by no longer requiring dephasing/rephasing gradients about the RF₉₀. In this configuration, more signals would be introduced outside the VOI in both the X and Y directions compared to only Y direction when a slice-selective RF₉₀ was used. While a two-step phase cycling would still be necessary to remove the signal outside the VOI, cycling out these higher outside signals would generally be more susceptible to subtraction artifacts, especially in human studies.

The change in spectral pattern as T_E increased from T_E = 20 to T_E = 100 ms was investigated using density matrix simulations¹⁹ for several strongly and weakly coupled metabolites such as Gln, GABA, GSH, and lactate (Lac). Spectra

where $O \left[\left(\frac{\pi J T_E}{2} \right)^2 \right]$ in Equation (1) denotes three-spin or higher coherences, $f_j(\tau) = \prod_{j \neq k} \cos(\pi J_{jk} \tau)$, $g_{jk}(\tau) = \sin(\pi J_{jk} \tau) \prod_{m \neq j,k} \cos(\pi J_{jm} \tau)$, and $\text{Signal}_{j,\text{in-phase}}^{\text{Refocused}} (T_E)$ and $\text{Signal}_{jk,\text{anti-phase}}^{\text{Refocused}} (T_E)$ are the in-phase and anti-phase contributions to the signal at T_E from the jth spin, respectively. For comparison, the corresponding in-phase and anti-phase contributions to the signal at T_E from the jth spin without the refocusing RF₉₀ are $\text{Signal}_{j,\text{in-phase}}^{\text{Unrefocused}} (T_E) = f_j(T_E)$ and $\text{Signal}_{jk,\text{anti-phase}}^{\text{Unrefocused}} (T_E) = g_{jk}(T_E)$, respectively.

In Equation (1), J-evolution is effectively refocused when $J_{jk} T_E \leq \frac{1}{3}$ for all spins j and k within a molecule since to first-order in $J_{jk} T_E$, $f_j \left(\frac{T_E}{2} \right) \approx 1$, $g_{jk} \left(\frac{T_E}{2} \right) \approx \frac{\pi J_{jk} T_E}{2}$, and thus

$Signal_{j,in-phase}^{Refocused}(T_E) \approx 1$ and $Signal_{jk,anti-phase}^{Refocused}(T_E) \approx 0$. Note that even for $J_{max}T_E \leq \frac{1}{2}$, $Signal_{jk,anti-phase}^{Refocused}(T_E)$ is still smaller than $Signal_{jk,anti-phase}^{Unrefocused}(T_E)$:

$$\begin{aligned} Signal_{jk,anti-phase}^{Refocused}(T_E) &= \left| \frac{\sin(\pi J_{jk}T_E)}{2} \left(\prod_{m \neq j,k} \cos^2\left(\frac{\pi J_{jm}T_E}{2}\right) - \prod_{m \neq j,k} \cos\left(\frac{\pi J_{jm}T_E}{2}\right) \cos\left(\frac{\pi J_{km}T_E}{2}\right) \right) \right| \\ &\leq \left| \sin(\pi J_{jk}T_E) \prod_{m \neq j,k} \cos(\pi J_{jk}T_E) \right| \end{aligned} \quad (2)$$

2.3 | In vivo measurements

Male Sprague-Dawley rats ($n = 3$) were studied using a horizontal-bore 9.4-T scanner (Magnex Scientific, Oxford, UK) interfaced to an Agilent DirectDrive console (Agilent Technologies, CA). Animals were anesthetized with 1.5% isoflurane in a mixture of 50% O₂:50% N₂O and placed in a cradle with the head fixed using a bite-bar and ear rods. Throughout the study, the animals' body temperature was monitored and maintained at 37°C using hot water circulation. All studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

In semi-LASER, a 1.0 ms Shinnar–Le Roux pulse (bandwidth = 13 kHz) was used for both excitation and for the RF₉₀ pulse, and 1.5 ms AFP pulses (HS1, bandwidth = 16.7 kHz) were used for refocusing. Water suppression (bandwidth = 270 Hz) was achieved using the VAPOR technique with interleaved outer-volume suppression pulses.²⁰ A home-built quadrature surface radiofrequency coil (two loops, 14 mm diameter each) was used as a transceiver.

MRS data were acquired from a 180 μ L VOI in the rat brain. At the start of the experiment, fast spin-echo images were acquired and used to position the VOI within tissue containing the hippocampus, thalamus and the midbrain. B₀ inhomogeneity in the selected VOI was adjusted using an adiabatic version of FASTESTMAP technique.²¹ The mean measured water linewidth using the real component was 18 Hz. Semi-LASER water-suppressed spectra (repetition time of 4 s, spectral width of 10 kHz) at T_E = 30 ms (32 averages) and T_E = 68 ms (128 averages) were acquired in an interleaved fashion switching between enabling and disabling the RF₉₀ pulse. Water reference scans were also acquired for quantification and for eddy-current correction. A T_E of 30 ms was considered since this is the shortest T_E typically achieved on the human clinical scanner at 3 T while a T_E of 68 ms was chosen to see the viability of using this *J*-refocused sequence for editing.

All MRS spectra were processed in MATLAB using MRSpa software.²² The data with and without RF₉₀ were

post-processed as two separate measurements. Frequency and phase corrections were performed followed by eddy current correction.²³ The resulting summed semi-LASER spectra (with and without an RF₉₀ pulse) were fitted using LCModel v6.3-0G.²⁴ Two basis sets, each consisting of 18 simulated spectra, were used as previously described⁸ together with the built-in macromolecular resonances in LCModel.

3 | RESULTS

Simulations of the total signal integral (with and without the refocusing RF₉₀ pulse) as a function of T_E for Gln, GABA, GSH, and Lac are shown in Figure 2. Even at the shortest T_E = 20 ms at 9.4 T, these metabolites underwent some *J*-modulation when the RF₉₀ pulse was not applied. Interestingly, more than 15% of the total signals were recovered with the refocusing pulse. As T_E increased, the signal integral decreased even though the spectral pattern was always positive. Compared to the other *J*-coupled metabolites in Figure 2, lactate, which is a weakly coupled spin system, exhibited relatively smaller *J*-modulation and changes in signal amplitude with and without an RF₉₀ pulse.

The effect of the refocusing RF₉₀ pulse on the spectral pattern at T_E = 30 ms is shown in Figure 2. As expected, the simulated spectrum with RF₉₀ pulse was much higher in intensity compared to the normal semi-LASER spectrum at the same T_E. For instance, the signal intensities for all resonances of Gln, GABA and Lac were at least twice as large with an RF₉₀ pulse compared to the case without an RF₉₀ pulse. Interestingly, the signal intensities from the cysteine and glutamate moieties in GSH, which are strongly coupled systems, were larger with an RF₉₀ pulse compared to the signal intensities in a glycine moiety, which is a weakly coupled system.²⁵

In vivo spectra acquired from a rat brain are shown in Figure 3. Visible differences in signal intensities were observed between spectra acquired with and without an RF₉₀ pulse, especially for Glu, Gln, GABA, and a multiplet of NAA (mNAA), which were consistent with the simulations in Figure 2. The effects of the RF₉₀ pulse were especially significant at longer T_E where larger *J*-modulation would be expected if not for application of the *J*-refocusing pulse. Moreover, *J*-modulation of the macromolecular resonances, particularly those at 0.89 and 1.70 ppm that are from *J*-coupled systems,²⁶ was refocused at both T_Es by application of an RF₉₀ pulse. Last, the intensity of the singlets of NAA, total creatine (tCr), and total choline (tCho) were unaffected by the *J*-refocusing pulse as expected. The signal-to-noise ratios (SNR) for tNAA peak measured for this animal were 99

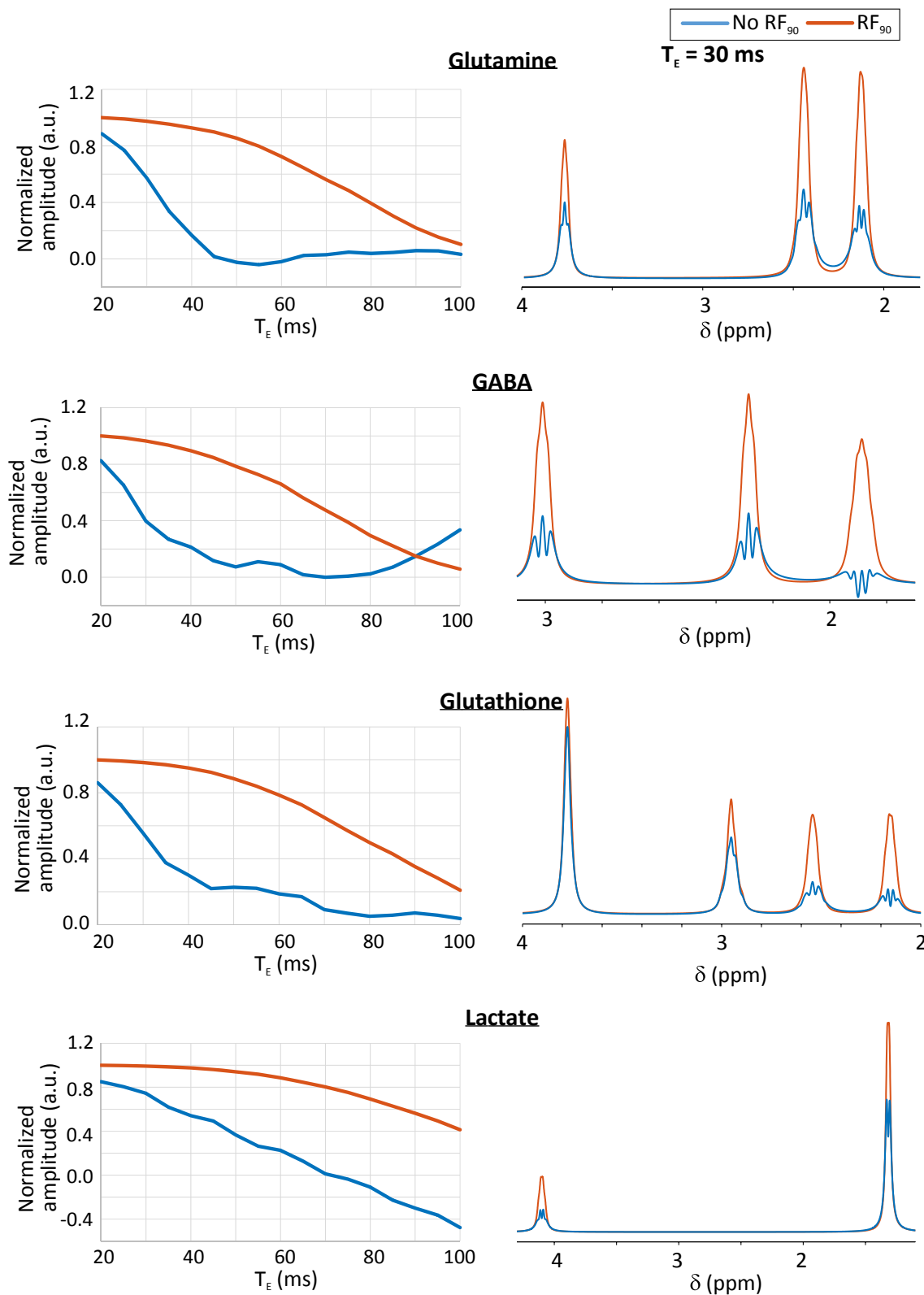


FIGURE 2 Left column, J -refocusing of Gln, GABA, GSH and Lac using semi-LASER at 9.4 T; standard semi-LASER (blue curve) and semi-LASER with an additional RF_{90} pulse (red curve). Signal integrals correspond to the amplitude of the first point of the simulated FID normalized to 1.0 at $T_E = 20$ ms when the RF_{90} pulse was enabled. T_2 relaxation and gradients were neglected in the simulation. Right column, Simulated spectra without (blue) and with (red) the RF_{90} pulse are shown at $T_E = 30$ ms. All spectra were line-broadened by 10 Hz for display purposes

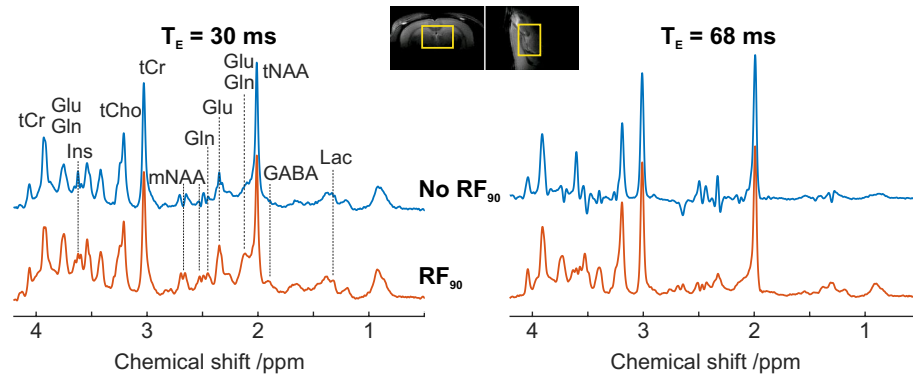


FIGURE 3 In vivo semi-LASER spectra acquired with (red) and without (blue) a J -refocusing RF_{90} pulse from one animal at $T_E = 30$ ms (left) and $T_E = 68$ ms (right). When an RF_{90} pulse was applied, the signal intensities from all J -coupled metabolites were higher compared to the case without an RF_{90} pulse. The macromolecular spectrum at both T_E s also changed with the inclusion of an RF_{90} pulse. All spectra were line broadened with a 1.5 Hz Gaussian line broadening for display purposes only. Insert shows the VOI location on axial and sagittal turbo spin-echo images. The dotted vertical lines are visual guides pointing to J -coupled metabolites in which J -refocusing is easily observed in the spectra

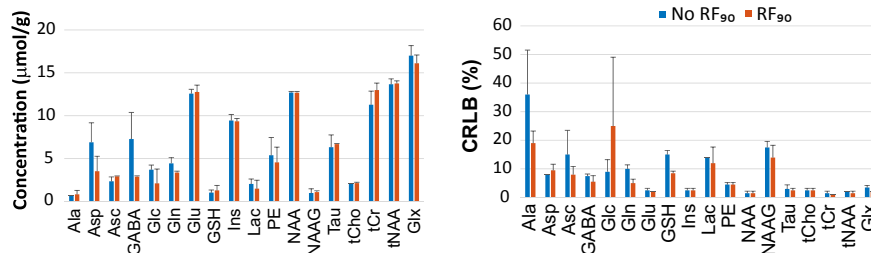


FIGURE 4 Neurochemical concentrations (left) and the corresponding CRLB (right) measured at $T_E = 30$ ms using the semi-LASER sequence with (red) and without (blue) an RF_{90} pulse. Error bars represent SDs between $n = 3$ animals. Ins, *myo*-inositol; PE, phosphoethanolamine; NAAG, *N*-acetyl-aspartyl-glutamate; Tau, taurine; tNAA, NAA+NAAG; Glx, glutamate+glutamine

vs. 96 at $T_E = 30$ ms and 152 vs. 146 at $T_E = 68$ ms without and with the RF_{90} pulse, respectively.

For $T_E = 30$ ms, the spectra with an RF_{90} pulse for J -coupled metabolites did not seem to undergo any significant J -modulation and were similar to the spectra acquired at the minimum achievable T_E of 19 ms (data not shown). However, the concentrations of almost all measured metabolites at $T_E = 30$ ms determined from the MR spectra with and without an RF_{90} pulse were similar as shown in Figure 4, with the exceptions of aspartate (Asp) and GABA. As a result of J -modulation, these metabolites were overestimated due to their out-of-phase J -modulated spectral pattern when no RF_{90} was applied, whereas the refocusing the J -modulation led to an in-phase spectral pattern that provided better quantification. Interestingly, a gain in quantification precision as reflected by the lower Cramer-Rao lower bound (CRLB) values was observed for alanine (Ala), ascorbate (Asc), GABA, Gln, GSH, and Lac as shown in Figure 4. Glucose (Glc) was the only metabolite where the CRLB was found to increase when using the J -refocused pulse.

At $T_E = 68$ ms, as the J -coupled metabolites underwent further J -modulation in addition to T_2 relaxation, the refocused signal intensities were lower compared to those

acquired at $T_E = 30$ ms with RF_{90} pulse as shown in Figure 3. For instance, the Gln signal integral was almost null at this T_E without an RF_{90} pulse but was almost 60% higher with an RF_{90} (Figure 3). However, the CRLB at $T_E = 68$ ms was comparable with and without the J -refocusing pulse (data not shown). This was most likely due to the distinct spectral patterns for Glu, Gln, and mNAA that were observed even without an RF_{90} pulse. Due to the small number of animals studied ($n = 3$), no statistical analysis was carried out.

Application of two slice-selective 90° pulses along the X-direction resulted in smoothed edges in the VOI profile along the X-direction as shown in Figure 5. Consequently, the VOI profiles in Y and Z direction were also lowered. Application of a J -refocusing pulse resulted in $\sim 10\%$ loss of water reference signal measured in vivo.

4 | DISCUSSION

The current study demonstrates that J -evolution of coupled metabolites can be refocused by inserting a 90° pulse with appropriate phase into the semi-LASER sequence. Since the majority of J -couplings for the metabolites studied in this

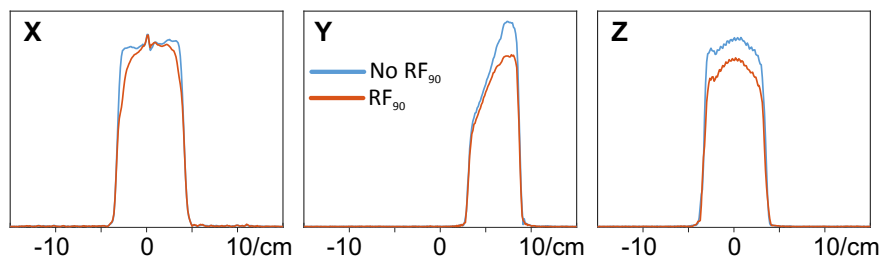


FIGURE 5 VOI profiles measured along the X, Y, and Z directions from a rat brain in vivo using the semi-LASER sequence ($T_E = 19$ ms, field of view = 3 cm, 256 complex points, spectral width = 100 kHz). Plots show the effect of an RF_{90} pulse on the VOI profiles (red curves with the RF_{90} pulse, and blue curves without the RF_{90} pulse). The profile along X-direction was smoothed on the edges due to applying two slice-selective 90° pulses

work ranged between 6 and 8 Hz, J -evolution was refocused using the J -refocused sequence at $T_E = 30$ ms since $\frac{\pi J T_E}{2} < \frac{\pi}{6}$. Since $0.64 \leq \frac{\pi J T_E}{2} \leq 0.86$ for $T_E = 68$ ms, however, some signal attenuation and J -modulation occurred although the RF_{90} pulse still reduced the overall amount of J -modulation since $J T_E \cong \frac{1}{4}$. With the proposed J -refocused sequence, a perfect spin-echo signal was measured at full signal intensity due to sandwiching the second RF_{90} between dephasing/rephasing gradients which resulted in an RF pulse with uniform phase applied throughout the sample. This is in contrast to a previous study¹¹ where 50% of the signal was lost (either dephased or placed along z-direction) due to sandwiching the second RF_{90} between spoiler gradients. The higher signal amplitudes due to suppressing J -modulation with an RF_{90} pulse imply higher SNR for Gln, GABA, and GSH, and this in turn leads to more reliable quantification precision of these metabolites. Although the novel sequence requires a two-step phase cycle to achieve good localization, the spectral patterns measured at relatively short $T_E = 30$ ms were comparable and only slightly lower in intensity due to T_2 relaxation to the spectrum acquired using the shortest achievable T_E .

One potential benefit of utilizing this novel sequence is for MRS studies where ultra-short T_E cannot be realized. For example, surface RF coils are typically used to achieve high transmit B_1 fields and SNR^{27,28} during MRS studies in animals. However, this only allows for investigating brain regions close to the surface and is dependent on the size of the surface coil. For deeper brain regions such as in the hypothalamus or pons in rodents, a volume RF coil, which has less B_1 efficiency than a surface coil, is generally required. Due to the poorer B_1 transmit efficiency of volume RF coils, longer pulse durations are required thereby increasing the minimum achievable T_E to times that are greater than 30 ms for pulse sequences such as semi-LASER or LASER.²⁹ A weak gradient coil might also prolong the minimum T_E since longer spoiler gradient durations would also be required to obtain good localization. In either case, the proposed MRS sequence would be useful since the spectral patterns under J -refocusing and measured at the minimal T_E with a volume coil would be comparable to the spectral patterns measured at ultra-short

T_E using a surface coil and will provide similar quantification accuracy.

Another potential advantage of using the J -refocused sequence is the increase in quantification precision of J -coupled metabolites at 3 T (e.g., Glu+Gln, GSH, *myo*-inositol) and 7 T (e.g., Gln, Glu, GSH, *myo*-inositol, taurine) in human studies when using relatively long T_E s of 25 to 40 ms. On clinical 3 T and 7 T MR scanners, volume coils (e.g., standard body coil or transverse electromagnetic [TEM] coil) are typically used whereby the minimal achievable T_E is around 30 ms with a semi-LASER sequence. With the additional RF_{90} pulse and dephase/rephasing gradients, this T_E would increase to about 35 ms. As mentioned above, the spectrum measured at this T_E would be comparable to ultra-short T_E data, thereby allowing for better quantification of coupled metabolites.³⁰ Although a gain in CRLB for J -coupled metabolites is expected when using this J -refocused sequence in human studies, the values are hard to predict based on animal data. This is because, in animals, the spectral linewidths are narrower and the T_2 relaxation times are longer compared to humans at a given field strength.³¹ The expected gain in CRLB also depends on several factors, such as bandwidth of RF pulses, inter-pulse delay, VOI dimension, number of averages used and the B_0 field strength used in the study.

While the current study used a semi-LASER sequence, the J -refocusing pulse can be easily inserted into other localized proton MRS sequences that contain refocusing pulses, such as PRESS³² and SPECIAL.³³ J -refocused PRESS might be a good alternative on animal scanners but is not recommended for human studies due to the larger chemical shift displacement error arising from the low bandwidth of the refocusing pulses.³⁴ For J -refocused SPECIAL, which uses two non-adiabatic 180° pulses, the phase cycle would have to be doubled to four scans to obtain 3D localization.³³ Note also that an RF_{90} pulse cannot be inserted between a pair of AFP pulses, such as those used in semi-adiabatic SPECIAL,³⁴ due to the quadratic phase generated under AFP pulses.

One downside of the proposed sequence is that the voxel shape is affected from the application of two gradient-selective 90° pulses. The selected 3D VOI is not cuboidal in

shape anymore but rather a cuboid with a smoothed edge in the direction where the RF₉₀ pulse is applied. As discussed earlier, sharper voxel shapes could be obtained using a single gradient-selective excitation pulse and a global RF₉₀ pulse for *J*-refocusing, although such modifications are more prone to subtraction artifacts outside the VOI. Even though inclusion of an RF₉₀ pulse led to a 10% loss in water signal, the gain in intensity for *J*-coupled metabolites outweighs the loss in VOI selection. Since both water reference and metabolite signals are acquired from the same VOI with the refocusing pulse, this should not affect metabolite quantification as demonstrated by the data in Figure 4.

5 | CONCLUSIONS

In summary, this study demonstrates the feasibility of acquiring ¹H MRS data with reduced *J*-modulation over a wide range of T_E. The proposed localized MRS sequence will be beneficial in both animal and human MRS studies when ultra-short T_E is not possible and will provide more reliable quantification precision for *J*-coupled metabolites. Future work includes assessing the proposed sequence in the human brain.

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