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Which pulse sequence is optimal for myo-Inositol detection at 3T?

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Abstract

Optimized myo-Inositol (mI) detection is important for diagnosing and monitoring a multitude of pathological conditions of the brain. Simulations are presented in this work, performed to decide which pulse sequence has the most significant advantage in terms of improving repeatability and accuracy of mI measurements at 3T over the pulse sequence used typically in the clinic, a TE=35ms PRESS sequence. Five classes of pulse sequences, four previously suggested for optimized mI detection (a short TE PRESS, a Carr-Purcell PRESS sequence, an optimized STEAM sequence, an optimized zero quantum filter), and one optimized for mI detection in this work (a single quantum filter) were compared to a standard, TE=35ms pulse sequence. While limiting the SNR of an acquisition to the equivalent SNR of a spectrum acquired in 5min from a 8cc voxel, it was found through simulations that the most repeatable mI measurements would be obtained with a Carr-Purcell sequence. This sequence was implemented in a clinical scanner, and improved mI measurements were demonstrated *in vivo*.

Keywords

myo-insitol; ^1H MRS; 3T; Monte Carlo simulations

Introduction

Myo-inositol (mI) is a ubiquitous cyclic sugar alcohol found in the brain. Although its function is not well understood, it is believed to be an essential requirement for cell growth, an osmolite, and a storage form for glucose (1). It has also been proposed as a glial marker (2). Altered levels of mI have been associated with multiple sclerosis (3) and brain injury (4). Moreover, increased levels of mI have been reported in mild cognitive impairment and Alzheimer's disease (5), suggesting it as potential marker for diagnosis and treatment of these widespread pathological conditions.

Although mI is present in normal brain at concentrations of up to 8mM (6), repeatable measurements of its concentration are difficult, and intra-volunteer coefficients of variation (CV's) only reach 6-8% (7,8). Usually, spectra for mI quantification are acquired in a clinical setting using a short echo time (TE) PRESS pulse sequence (TE=30 or 35ms are typical (9)). Much of the limited mI measurement reproducibility is due to its the low signal to noise ratio (as the mI signal is split between six coupled protons), as well as to its spectral overlap with a number of other brain metabolites, including glutamate (Glu), glutamine (Gln), glycine (Gly), taurine (Tau), choline (Cho) and macromolecules (MM's). This overlap of multiple, complicated spectral lines introduce uncertainty in the fitting process, needed to extract metabolite concentrations.

A number of acquisition strategies has been proposed in the past to increase the reproducibility of mI measurements (10-14). These strategies have two fundamental modes of action: selectively boost the mI signal (usually by reducing mI signal evolution under J coupling), or selectively reduce the overlapping, background resonances. While all of these strategies have compelling arguments in their favor, they also have flaws. For example, it is difficult to increase the mI signal without also increasing the signal of the overlapping resonances. It is also difficult to decrease the background signal without also decreasing the mI signal to levels that make its quantification difficult. It is therefore not immediately straightforward to decide which of the proposed approaches yields the most accurate and reproducible mI measurements.

The definite answer for this question would be provided by experimental data acquired *in vivo* with the multiple pulse sequences suspected to provide better mI measurements. Obtaining this answer, however, is impractical for a number of reasons. First, it is not trivial to implement a large number of specialized pulse sequences on a clinical scanner. Secondly, it is difficult to acquire a large number of data sets from human subjects from the same voxel, to provide a direct comparison between pulse sequence performances. Most importantly, however, the ground truth of the absolute mI concentration is never known for *in vivo* measurements, leading to the impossibility of assessing pulse sequences for accuracy.

It is the intent of the work proposed here to perform simulations and decide which pulse sequence has the most significant advantage in terms of improving repeatability and accuracy of mI measurements. The response of 14 metabolites found in the human brain to a number of pulse sequences is computed using the GAMMA libraries (15). Pulse sequences considered in our simulations include a standard, TE=35 PRESS pulse sequence (which we define as the clinical standard), a very short TE PRESS pulse sequence (13), a Carr-Purcell echo train (CPRESS) with a varying number of refocusing pulses (11,16), an optimized STEAM sequence (14), and a zero quantum filter (ZQF) (10). All of these sequences have been proposed in the past as potential candidates for optimized mI detection. Additionally, a single quantum filter (SQF), initially proposed for better Tau detection (17), and whose timings were numerically optimized in this work for better mI detection, was also considered in the simulations. For each of the pulse sequences considered, the 14 individual spectra resulting from the GAMMA simulations, weighted according to their reported *in vivo* concentration, together with simulated residual water and macromolecule signals are added together to simulate a human brain. Noise is then added to the resulting "brain" signal, and the data is fit using LCModel (18). The process is repeated 1000 times for each pulse sequence, while using different noise seeds; the resulting fitted mI concentration is saved for each run. Two separate noise levels are considered in our simulations: one corresponding to a standard clinical acquisition (a 5 min acquisition from a 8cc voxel) and the second one corresponding to double the signal to noise (SNR) of the standard clinical acquisition. Statistical comparisons were performed to decide whether differences in measurement variance are statistically significant. The most promising pulse sequence is implemented in a 3T clinical scanner, and improved mI measurement repeatability is demonstrated for this pulse sequence *in vivo*.

Methods

The response of each metabolite to a pulse sequence was computed using the GAMMA set of libraries. These libraries use a density matrix description of the spin system, and provide an object oriented programming approach for the simulation of NMR experiments (15). For each of the studied metabolites, an input file, containing the number of spins, their chemical shifts and the couplings between the different spins of the system is initially constructed. For our simulations, data published in (6) is used for these input parameters. During the free evolution periods, the spins evolve under the sum of the isotropic Hamiltonian and the J coupling Hamiltonian. RF pulses are considered ideal in our simulations, evolving the system under the

limit that the applied pulse is infinitely strong and infinitely short; all spins affected by this pulse are rotated by the same angle and phase. For a typical clinical acquisition, such ideal pulses have been demonstrated to offer a good compromise between computational speed and obtaining a good spectral response over the explicit consideration of frequency selective and gradient pulses (19). Separate pseudocodes, containing the explicit timing of the RF pulses/free evolution times, were written using the GAMMA libraries for each of the pulse sequences considered.

The diagrams and timing denominations of the five separate types of acquisitions considered in our simulations (PRESS, CPRESS, STEAM, ZQF and SQF pulse sequences) are depicted in Figure 1. All the RF pulses depicted in Figure 1 as hard pulses in colors other than white (grey or black) are meant to be slice selective, and have gradients associated with them, not depicted in Figure 1 for simplicity. In practice, for the STEAM and PRESS, the 90° pulses were implemented in our clinical scanner as 3.5ms sinc pulses, with 2400Hz bandwidth, and the 180° pulses as 6.5ms sinc pulses, with 1100Hz bandwidth. Ten separate pulse sequences were considered within these five classes as following:

1. PRESS, TE=35ms, $t_1=7$ ms (this is our standard clinical pulse sequence)
2. PRESS, TE=15ms, $t_1=3.8$ ms (this is the close to the potential minimum timing achievable for a PRESS sequence on our clinical 3T scanner, while using a standard head coil)
3. CPRESS with 2 additional refocusing pulses (CPRESS 2), TE=45ms
4. CPRESS with 4 additional refocusing pulses (CPRESS 4), TE=67ms
5. CPRESS with 6 additional refocusing pulses (CPRESS 6), TE=89ms

For the CPRESS acquisitions, the timing between the first 90° degree pulse and the first 180° degree pulse, t_1 , was kept to the minimum allowable by the scanner (3.8ms). The timing between the 180° degree pulses, $t_{cp}=TE/4$, is also kept to the minimum achievable by our 3T clinical scanner, of 11.2 ms. Additionally, although simulated as ideal 180° degree pulses, for the clinical implementation, the two additional refocusing pulses (depicted in black in Figure 1b) are implemented as quadratic phase 180° degree pulses, of 2.01ms duration and 2500Hz bandwidth (12). These pulses were designed using the Shinnar-LeRoux transform (20). In order to increase the RF pulse bandwidth without a penalty in peak B_1 , non-linear phase was created across the spin-echo profile. This was accomplished by using the “root-flipping” method (21).

6. STEAM, TE=5ms, TM=5ms. This is close to the minimum timing achievable on a clinical scanner (22), providing maximum yield for the mI signal (14)
7. STEAM, TE=180ms, TM=40ms. This is the timing of the pulse sequence found through simulations to offer the best signal to background ratio for the mI signal (14)
8. ZQF, TE=89ms, $TE_1/TM/TE_2 = 50/9/30$ ms respectively. This is the timing of the pulse sequence previously demonstrated to maximize the mI signal (10).
9. ZQF, TE=114ms, $TE_1/TM/TE_2 = 75/9/30$ ms respectively. This is the timing of the pulse sequence previously demonstrated to maximize the ratio of the mI to background signals (10).
10. SQF, TE=90ms, $t_1=t_2=22.5$ ms. The timing of the pulse sequence was numerically optimized in this work. The mI signal was simulated through this pulse sequence, while varying the TE (while keeping $t_1=t_2$) and noting the timing for which the mI signal was maximized.

The only gradients simulated in this work are the G_1 gradients following the first and third pulse of the STEAM sequence, and the G_2 gradients applied during the TM period of the STEAM sequence (Figure 1c) and of the ZQF filter (Figure 1d). The action of these gradients was simulated indirectly (therefore the strength of these gradients, G_1 and G_2 , and their duration, t_1 and t_2 , is irrelevant), in a manner identical to the one described in (19). In essence, four separate simulations were performed for the STEAM sequence, consisting of an incremental rotation of $\pi/2$ about the z axis, following the first and third RF pulses. These 4 simulations were averaged at the end. It has also been assumed that only the elements of the density matrix corresponding to the zero quantum coherences (which are insensitive to the applied gradient) remain non-zero at the end of the TM period during both STEAM and the ZQF. For more details regarding the simulations of these gradients, the reader is referred to (19).

Relaxation was not explicitly included in the simulations. A penalty factor of $\exp(-TE/T_2)$, however, was multiplied to all time-domain data, to account for signal loss in longer TE sequences. Here T_2 is the transverse relaxation time; consistent with published literature reports (23), the T_2 values used for all the metabolites and all the pulse sequences apart from CPRESS were 250ms. MM's T_2 's were 35ms. Also consistent with literature reports (16), for CPRESS the metabolite T_2 's were 375ms, and the MM's T_2 's were 75ms.

Metabolites included in our simulations, listed here together with their concentrations are N-acetyl aspartate (NAA) [12mM], phospho-choline (Cho) [2mM], creatine (Cr) [7mM], phospho-creatine (PCr) [3mM], Glu [10mM], Gln [4.5mM], Tau [1.2mM], ml [6mM], lactate (Lac) [0.4mM], Gly [0.7mM], aspartate (Asp) [1.2mM], alanine (Ala) [0.8mM], gamma amino butyric acid GABA [1.6mM] and guanidine (Gua) [0.2mM].

For each of the pulse sequences considered, and each metabolite, simulations were initially run, and resulting spectra were line-broadened to 3Hz for construction of LCModel basis set spectra. Subsequently, a set of spectra line-broadened to 7 Hz was generated, providing more realistic simulations for *in vivo* acquisitions. The 14 resulting spectra were added together with weights corresponding to their *in vivo* concentrations. Additionally, a residual water signal, and a simulated MM signal were added to the simulated brain spectrum. The simulated MM signal was designed to be similar to MM signals acquired *in vivo* under similar conditions (16). More precisely, the simulated MM signal was a sum of 4 Gaussian signals, centered at 3.9, 2.15, 1.45 and 0.9ppm, of widths 0.8, 1.3, 1 and 0.8ppm, respectively. The relative MM peak heights were in the ratio 1:2:4:2. The absolute scaling of the MM signals was such as the ratio of the NAA peak to the 1.45ppm MM signal was ~ 5 for the PRESS, TE=35ms pulse sequence, somewhat typical of an *in vivo* acquisition. The MM signal added to the simulated metabolite signals was scaled according to the echo time of the pulse sequence and the MM T_2 . Although this MM signal may differ to a certain extent from individual *in vivo* MM signals, it represents a good approximation and can give good insight into the effect of MM signals on signal quantification.

Following the generation of a noiseless brain spectrum for each of the pulse sequences considered, noise was added with a given standard deviation σ . Two distinct sets of simulations were performed for each pulse sequence. In the first run, the equivalent SNR of the spectrum (defined as the maximum signal in the spectrum minus the baseline signal divided by 2σ) was the one of a clinical PRESS, TE=35ms acquisition, acquired in 5 minutes (TR=2s), from an 8cc voxel (SNR ~ 22). In the second set of simulations, double the clinical SNR was used. It is estimated that this SNR level is close to the upper end of what can be achieved in a clinical setting at 3T, by using a combination of larger voxels, increased averaging, and the use of local receive coils (if the voxel studied is in an anatomical location where such coils can offer an

increased SNR). Note that, for a given SNR level, the standard deviation of the noise added was kept constant between the different pulse sequences studied.

The “noised” brain spectra were then fitted using LCModel, and the metabolite concentrations were recorded for each run. The process was repeated 1000 times for each pulse sequence (and for each SNR level), with different noise seeds. Averages and standard deviations were computed for the metabolites of interest. Absolute scaling of the spectrum was performed such as the average NAA concentration for each particular pulse sequence was calibrated to its known level (12mM). This is equivalent to obtaining the overall calibration factor f_{calib} needed for LCModel using NAA phantom measurements. For the pulse sequences purposely suppressing the NAA singlet (all multiple quantum filters), however, an average calibration factor f_{calib} from all the other pulse sequences was used, resulting in approximate “absolute” error measurements. The difference between the smallest f_{calib} and the largest f_{calib} factor for all pulse sequences was $\sim 5\%$.

Although concerns might arise regarding the creation of over-idealized conditions by fitting simulated data with simulated basis sets, and hinting to the potentially more preferable solution of fitting simulated data with experimentally measured basis sets, a recent literature report allays this concern (24). This report demonstrated that while fitting measured data with simulated basis sets, and fitting simulated data with measured basis sets might result in slightly biased concentration measurements for certain metabolites (but not mI), the uncertainty of the measurements in the two cases is not different than the uncertainty of measurements obtained by fitting measured data with measured basis sets and simulated data with simulated basis sets. We have therefore resorted to the much easier to implement solution of fitting the simulated brains with simulated basis sets.

The Monte Carlo simulation approach used in this work to decide which is the most promising acquisition strategy for mI detection, although previously used in a different context (24), is uncommon. Most typically, the yield of a newly proposed pulse sequence is computed, and compared to the yield of a standard acquisition (10). The computation of a yield factor, however, does not traditionally account for the additional signal loss due to transverse relaxation decay. Most importantly, however, the yield factor does not immediately translate into the relevant parameter of interest, measurement repeatability. Repeatability is a complex function of the yield of all metabolites in the spectra, the SNR of a given acquisition, the fitting subroutine used, etc. The proposed Monte Carlo simulation approach used here was chosen over the standard, yield computation approach because it results in the direct computation of a measurement repeatability, while using the same fitting program to be used *in vivo*, and accounting for the SNR of the acquisition (which, as demonstrated later in the manuscript, may impact various pulse sequences differently).

Levene's homogeneity of variance test was performed to decide whether differences in measurement variance are statistically significant. The pulse sequence providing the most repeatable mI measurements, while also maintaining measurement accuracy, was implemented in a 3T, GE clinical scanner. A 600ml spherical phantom, containing 200mM mI (Fluka 57570), with its pH adjusted to 7.2, was initially scanned to insure a proper match between our simulations and the experimental data acquired using PRESS and STEAM. Three volunteers (average age 34 years) were subjected to a one hour exam, in which five PRESS, TE=35 acquisitions, and five acquisitions using the most promising mI measurement sequence (one of the 8 pulse sequences listed above). All 10 spectroscopic measurements were acquired from an 8cc voxel situated in the posterior cingulate gyrus, and had TR=2s, and 128 averages, and a total acquisition time of ~ 5 minutes. All *in vivo* exams were performed under a protocol approved by the Institutional Review Board. Cramer Rao Lower Bounds (CRLB's) were reported for both sequences as a measure of repeatability (25). To best match simulation and

experimental data, “within session” (and not “between-session”) repeatability measures were chosen here to demonstrate pulse sequence performance. Given the limited experimental data on the relative contribution of the “between session” variance to the total variance of MRS experiments, changing from significant (for NAA, e.g.), to completely insignificant (for mI) (7), it is felt that achieving accurate, experimentally validated modeling of effects related to removing and repositioning the patient in the scanner (such as changes in coil loading, voxel repositioning, water suppression) would be difficult to achieve, and was not attempted in this work. It is highly probable that the majority of the effects related to removing and repositioning the subjects in the scanner (as well as patient motion, which was not modeled here, either) will impact most of the (single shot) pulse sequences considered here in a similar manner, and may result in a decrease in the repeatability of the data acquired with all the pulse sequences considered.

Results

Figure 2 and 3 demonstrate a validation of the performance of our simulations tools. Figure 2a represents a fit of the experimental data acquired from our 50mM mI phantom, using JPRESS (TE=35-192.5), (grey dashed line) and a simulation using the same experimental pulse sequence parameters (black line). Figure 2b presents the same comparison for the CPRESS2 pulse sequence. Given the slight differences between our experimental conditions and the conditions reported in (ref-mI6), which provided the chemical shifts and J couplings for our simulations (the pH of our phantom was 7.2, vs. 7 in (ref-mI6), and the temperature of our phantom was 24°C vs 37°C in (ref mI 6)), the agreement between the experimental data and simulations is convincing. Figures 3a and 3b, respectively, represent simulated brain spectra for the JPRESS, TE=35-192.5ms pulse sequence, and for the CPRESS2 pulse sequence, along with the LCModel fit. Figures 3c and 3d displays spectra acquired *in vivo* from a normal volunteer using the same sequences, along with the LCModel fit. As observed from the matches between Figures 3a and 3c, and Figures 3b and 3d, respectively, there is a close resemblance between the simulated the *in vivo* data.

Table 1 presents a measure of repeatability (the coefficient of variation expressed as a percentage, %CV) and accuracy (defined as the average measured concentration minus the known input concentration divided by the known input concentration) for (mI+Gly) and mI levels, for all ten pulse sequences considered at the clinical SNR level. The average CRLB's yielded by LCModel are also presented in this table. The results presented as N/A were not displayed due to the very limited SNR available for proper spectral quantification. Table 2 presents the same results as in Table 1, at double the SNR level. A few salient general characteristics of these tables include the following:

- Consistent with previous literature reports (26), increased measurement repeatability is associated with increased SNR
- The mI and Gly signals are not distinguishable from each other, unless a multiple quantum filter is used to remove the Gly signal. Unless strong evidence exists that in a certain disease when mI signal is known to change, Gly is also changing, much more accurate detection of mI + Gly is possible
- The multiple quantum filters reduce the undesired, background signals, but they also reduce the mI signal to low or very low levels. Among the variants, the optimized SQF appears the better choice for uncontaminated mI detection.
- Repeatability regarded as a single measure of pulse sequence performance is not adequate; accuracy also needs to be factored in. For example, increased mI measurement performance might be asserted for the 4 and 6 pulse CPRESS sequences of Table 1 and

2, while in effect increased mI repeatability is only obtained at the expense of larger absolute error (ie mI is overestimated, while Gly is underestimated).

- The “measured” %SD are lower than the CRLB's by ~5-35% This may be explained by the fact that estimated CRLB's (which may be biased by the presence of noise and the assumption of a potentially imperfect fitting model), and not theoretical CRLB's are reported in this manuscript. In general, the CRLB's are a particularly valuable measure of repeatability *in vivo*, when obtaining a large number of repetitions to compute an accurate coefficient of variation is difficult (25).

Levene's homogeneity of variance test was performed in order to verify if statistical differences exist between the variances of the metabolite concentration measurements. For the clinical SNR level, for the (mI +Gly) concentration (the only one that can be measured relatively repeatably at this SNR level), the following results were obtained: while reduced TE PRESS sequence offers slightly higher repeatability than the standard TE=35ms PRESS, the reduced measurement standard deviation is not statistically significant. At the same time, however, the 2 and 4 echo Carr Purcell sequences offer smaller measurement variance (p value for Levene test $p < 10^{-3}$). There is no significant difference between the variances of the 2 and 4 pulse CPRESS sequences. The relatively increased variance of the CPRESS 6 sequence is estimated to be due to lower spectrum SNR, due to increased TE. Also note that STEAM decreases repeatability of the mI measurements compared to the standard PRESS sequence, in both the very short TE case (TE/TM=5/5ms), and the optimized signal/background version (TE/TM=180/40ms). This is most probably due to the reduced SNR of STEAM (a factor of 2 compared to a PRESS sequence with similar echo time).

For the simulations performed at twice the clinical SNR level, the results (displayed in Table 2) were the following: for the (mI+Gly) concentration, both the PRESS, TE=15, the CPRESS 2 and CPRESS 4 variances were smaller than the one of the PRESS, TE=35 pulse sequence ($p < 10^{-3}$). The variances of both the CPRESS 2 and CPRESS 4 were also smaller than the variance of the short TE PRESS sequence ($p < 10^{-3}$). For the mI concentration, the variance of the optimized SQF was smaller than the variances of any other pulse sequence ($p < 10^{-3}$).

Consequently, we have decided that, at a clinical SNR level, a CPRESS sequence offers the best compromise between measurement repeatability and accuracy, at least in the case when the compromise of measuring the (mI+Gly) levels is acceptable. This sequence, with 2 additional refocusing pulses (TE=45ms), was implemented in our 3T clinical scanner. Illustrative *in vivo* spectra acquired with this sequence, as well as with PRESS, TE=35ms, from the same voxel of the same volunteer, at the same acquisition time of ~5minutes are displayed in Figure 4a and 4b, respectively. The average CRLB's from our small pool of measurement in human volunteers are 5% for the TE=45ms, CPRESS sequence, and 6.2% for the PRESS sequence, confirming the improvement measurement repeatability predicted by our simulations.

Discussion

Simulations were performed to understand the characteristics of pulse sequences that would provide optimal detection for mI at 3T. Direct comparisons were performed between five classes of pulse sequences potentially promising for improved mI detection. Four of these approaches were previously proposed for improved mI detection (short TE PRESS, CPRESS, STEAM and ZQF), while the fifth one, and an optimized SQF, had its timings optimized for better mI detection in this work.

Two dimensional experiments may also theoretically allow unbiased mI measurements. Variants of L-COSY or J-PRESS (27) have been sporadically been used in the past in an attempt

to obtain better, unbiased mI measurements in the brain. While arguments may exist on why such approaches would offer better sensitivity than the n-quantum filters in NMR experiments (28), practical aspects of implementing such approaches in clinical scanners (such as relatively large minimum echo times, and a low resolution in the second dimension, to keep acquisition time manageable) have rendered the use of these techniques questionable for routine MRS acquisitions. The reduced repeatability of such measurements reported *in vitro* (mI CV's of ~20% reported in Table 2 of (29)) and *in vivo* (30,31) (mI CV's in excess of 10% reported from data acquired with an SNR level of at least 4 times higher than the highest SNR considered in this report) have led the author to exclude such approaches from the current report. It is also questionable how efficient of a separation between Gly and mI is achievable with these acquisitions, while keeping the acquisition time manageable; overestimation of Gly by 160% and underestimation of mI by ~20% have been reported in a brain phantom (29), indicating improper separation of the two metabolites. Should it be desired, however, simulations of such 2D acquisitions can also be performed in a similar manner to the ones described here. It is probably recommended, however, to use a 2D fitting approach similar to the ones described in (29,32) instead of LCModel for data quantification.

A few conclusions can be drawn from the results displayed in Table 1 and 2. At the clinical SNR level, the mI concentration (as opposed to the mI+Gly concentration) cannot be measured accurately and repeatably. It is only the multiple quantum filters that suppress the singlets in the spectrum, including Gly, allowing unambiguous detection of mI. Such filters, however, decrease the (limited) SNR existent in a clinical scan to a degree almost unrecoverable, and may end up having the opposite effect to the one for which they have been designed, ie decreased performance in mI detection (note the results for the ZQF optimized for mI/background ratio). Increased SNR, however, which may be obtained by a reasonable combination of larger voxel studied, increased scan time, and use of local reception coils, can bring mI measurements to a point at which they can be valid diagnostic or treatment monitoring points even on an individual basis. The single quantum filter optimized in this work for mI detection is the one pulse sequence among the ones considered by us which can offer the best outcome.

It is not clear, however, how important it is clinically to obtain untainted measurements of the mI concentration alone. The presence of Gly, whose 3.55 resonance overlaps with mI, makes separate quantification of these two metabolites very difficult. Gly is an inhibitory neurotransmitter and antioxidant present in the brain at levels of 0.5-1mM (6). A limited number of technical studies have been published to date (33,34) attempting to tailor pulse sequences for improved Gly detection *in vivo*. Relatively large measurement errors of ~15% for Gly measurements are reported even in such tailored approaches. Yet fewer studies exist quantifying Gly levels in pathological conditions, and, to the author's best knowledge, it is only studies related to two relatively common conditions, hyperglycemia and certain types of tumors (35,36), reporting unambiguous changes in Gly levels. For the majority of clinical studies including ¹H MRS scans, no information exists regarding Gly levels. Unless it is known that the Gly concentration changes in the opposite direction to the mI concentration for a given pathological condition studied, it is preferable that combined (mI+Gly) detection be performed. Assuming that two categories of people (normal and diseased) are to be separated in a clinical trial, eg, and that a normal, 5 minute MRS examination is to be performed on each subject to distinguish between the 2 classes of subjects, power calculations strongly support this statement. Assuming that Gly concentration remains unchanged in the disease, a 10% increase in the mI concentration results in ~9% increase in the (mI+Gly) concentration. At ~3.3% error in measuring the (mI+Gly) concentration (characteristic for a CPRESS 2, clinical pulse sequence), 5 patients would need to be enrolled in group to detect the difference between the two categories of subjects at a power level of 0.95, based on the (mI+Gly) levels. Should the same difference be sought after while using the best mI measurement available (at the 7.5%

error of the SQF for clinical SNR), 15 people would need to be enrolled in the trial to be able to distinguish between the 2 categories of people based in mI levels alone.

Among the pulse sequences considered by us, the Carr-Purcell echo train is the one offering the best performance in (mI+Gly) detection. Although it is not entirely clear why it performs better than the short TE PRESS sequence, there are arguments in its favor. The short TE PRESS sequence increases both the mI signal and its overlapping resonances (MM's, Glu, Gln, Tau, Gly, etc), resulting in higher mI SNR, but more difficult quantification. As a result, it is only a more modest improvement that is obtained while using this sequence. In opposition, the CPRESS sequence employs a longer echo time, and maintains the MM signals at a level comparable to (or smaller than) the ones in the PRESS sequence. Due to the particular spectral pattern of Glu and Gln, some of the most important neighbors of mI, the ~ 3.7 ppm resonances of these metabolites are attenuated significantly more than the mI resonances (Figure 3). As previously mentioned (11,37), only weakly coupled spin systems undergo a modulation with frequency $1/2J$. The amplitude modulation depends on a factor R_{tcp} , with $R = \sqrt{\delta^2 + J^2}$, where J is the coupling constant and δ represents the chemical shift difference between the coupled multiplets. As the factor R is larger for the Glu and Gln resonances at ~ 3.7 ppm due to larger chemical shifts between the coupled multiplets (and similar J couplings with the mI protons), the signals from these resonances are attenuated faster than the ones from the mI spectrum. It is also probably the attenuation of these signals that lead to a small overestimation of mI with CPRESS compared to a standard PRESS (note the slightly larger absolute errors with CPRESS in Tables 1 and 2), probably at the expense of underestimating Glu and Gln.

It is important to state that these simulations are somewhat dependent on a few parameters, such as the MM and metabolite T_2 's, and the particular strength of MM signals. Shorter metabolite T_2 's tend to preferentially penalize longer TE sequences, while higher background MM signals decreases the repeatability of the measurements with all pulse sequences. Unless very large differences between the values considered by us, and the values of these parameters measured in a given *in vivo* scan, the sensitivity of the results to these parameters is not overwhelmingly significant. To verify that we have not accidentally favored the CPRESS sequences by using larger metabolite T_2 's than for the other sequences, for the clinical SNR case, we repeated our simulations for the CPRESS 2, while using metabolite T_2 's of 250ms, and MM T_2 's of 35ms (the same as the ones used for the other pulse sequences, but significantly lower than the published values for a CPRESS sequence (16)). The results were comparable, resulting in a CRLB of 4.3%, and %SD of 3.2%. Doubling the MM signals in the 250ms/35ms simulations resulted in increasing the CRLB's to 4.7% and %SD to 3.4%. We can therefore conclude that our results will probably hold for a large array of *in vivo* situations.

The results of our limited *in vivo* scans are very close to the ones predicted by simulations. The simulated basis sets fit the *in vivo* data sets very well for both pulse sequences (note the residuals in Figure 4); the CRLB's predicted by the simulations of 6.1% and 4.3% for simulations for PRESS, TE=35ms and CPRESS2, respectively translate *in vivo* to 6.2% and 5%, respectively. While the match between the predicted CRLB's and measured CRLB's is excellent for PRESS, slightly larger CRLB's result from fits of experimental data than from simulations for CPRESS2. It is possible that this effect is due to the shape of the extra two refocusing pulses. Due to the limited B_1 available on our clinical head coil, and to keep the duration of the extra 180 degree pulses short, these two pulses were implemented as quadratic phase pulses (12). This approach may increase the intensity of unwanted coherences (11) (effect not considered in our simulations), and result in higher overall measurement variability. While using a smaller coil, offering a higher B_1 per unit current, we are in the process of comparing the current results with the ones obtained using sinc refocusing pulses.

In the same context, a few additional remarks need to be made regarding the limitations of the simulations described in this work. Firstly, RF pulses were simulated here as ideal, rotating all the spins in the sample by a given angle. Different sequences might be particularly sensitive to the particular shape of the excitation pulses. For example, as demonstrated in (38), selective 180 degree pulses, such as the ones needed in PRESS, are usually far from ideal, their coherence transfer matrices depend on their shape, and the resulting level of coherence proliferation in PRESS may be far greater than resulting from the selective 90 degree pulses of STEAM. Care must be taken to insure that the particular implementation of the RF pulses in a clinical scanner result in signals that are well approximated by the simulations (see the good match between the simulated and experimental data in Figure 2a), otherwise the exact shape of the pulse might need to be included in the simulations. Secondly, our simulations only include “ideal” flip angles. While certain pulse sequences (such as PRESS) might be quite tolerant to small deviations from prescribed pulse angles, other sequences (usually the multiple quantum filters) are much more sensitive to those, and might result in drastic signal loss or improper selection of coherences, should such RF pulses not have their intended flip angle in the experiment. Last, but not least, our simulation were performed for 3T, and are only valid for this field strength. It is not unlikely that stronger field strengths such as 7T might provide higher SNR's which could propel the multiple quantum filters higher up in the list of pulse sequences to be used for improved mI detection.

Conclusions

This work presents simulations performed in order to decide which pulse sequence has the most significant advantage in terms of improving repeatability and accuracy of mI measurements at 3T. Five classes of pulse sequences, four previously suggested for optimized mI detection (a short TE PRESS, a Carr-Purcell PRESS sequence, a STEAM sequence, and an optimized zero quantum filter), and one optimized for mI detection in this work (a single quantum filter) were compared to a standard, TE=35ms pulse sequence. The results of the simulations, indicating more repeatable mI measurements with a Carr-Purcell sequence, were demonstrated *in vivo*.

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List of Abbreviations

mI	myo-inositol
NAA	N-acetyl aspartate
Gly	glycine
Cho	choline
Tau	taurine
Glu	glutamate
Gln	glutamine
Cr	creatine
PCr	phospho creatine
Lac	lactate
Asp	aspartate
Ala	alanine
GABA	gamma amino butyric acid
Gua	guanidine

%SD	percent standard deviation
CRLB	Cramer Rao Lower Bounds
PRESS	Point resolved spectroscopy
JPRESS	J resolved point resolved spectroscopy
L-COSY	Localized 2D correlated MR spectroscopy
STEAM	Stimulated echo acquisition mode
CPRESS	Carr Purcell PRESS
ZQF	zero quantum filter
SQF	single quantum filter
MM	macromolecule

Figure 1.

Classes of pulse sequences and their timings used in our simulations. **a)** PRESS pulse sequence **b)** Carr Purcell (CPRESS) pulse sequence with two extra refocusing pulses (note that 4 and 6 extra refocusing pulses were also considered, by inserting two or four additional t_{cp} intervals prior to the final z slice select pulse) **c)** STEAM sequence **d)** Zero quantum filter (ZQF) and **e)** Single quantum filter (SQF).

Figure 2.

Validation of the performance of the simulation tools. **a)** Simulated (straight gray line) and experimentally acquired data (dashed black line) from a 200mM mI phantom using PRESS, TE=35ms **b)** Simulated brain data set using a PRESS, TE=35ms pulse sequence **c)** Illustrative data set acquired from the brain of a normal volunteer with a PRESS, TE=35ms pulse sequence.

Figure 3.

Simulated brain data sets and fits for **a)** PRESS, TE=35ms pulse sequence **b)** PRESS, TE=15ms **c)** CPRESS, 2 extra refocusing pulses, TE=45ms **d)** CPRESS, 4 extra refocusing pulses, TE=67ms **e)** CPRESS, 6 extra refocusing pulses, TE=89ms **f)** STEAM, TE/TM=5/5ms **g)** STEAM, TE/TM=180/40ms **h)** ZQF, TE₁/TM/TE₂=50/9/30ms **i)** ZQF, TE₁/TM/TE₂=75/9/30ms **j)** SQF, TE=90ms. All figures are displayed at double the SNR level. The solid lines underlining the spectra represent the baselines, and the arrows point to the ml signal in all spectra.

Figure 4.

In vivo data sets, acquired using **a**) CPRESS with two extra refocusing pulses, TE=45ms and **b**) PRESS, TE=35ms. The mI signal is highlighted by arrows.

Table 1

Results for simulations performed at the clinical SNR level. The percent coefficient of variation (%CV), the Cramer-Rao lower bounds (CRLB's), and absolute error (expressed as a percentage) are presented for both the ml and (ml+Gly) measurements for the ten pulse sequences considered.

Pulse Sequence	% CV (ml+Gly)	CRLB's [%]	absolute error (ml+Gly) [%]	% CV ml	CRLB's [%]	absolute error ml [%]
PRESS						
TE=35ms	4.6%	6.1%	-0.6%	13.9%	19.3%	-19.3%
TE=15ms	4.2%	5.1%	1.2%	8.3%	10.9%	-4.6%
Carr Purcell echo train						
CPRESS 2 (TE=45ms)	3.3%	4.3%	2.8%	8.4%	11.7%	-3.2%
CPRESS 4 (TE=67ms)	3.0%	4.1%	5.8%	4.9%	6.9%	14.2%
CPRESS 6 (TE=89ms)	3.8%	5.1%	4.5%	5.8%	8.4%	12.8%
STEAM						
TE/TM=5/5ms	6.8%	8.4%	1.8%	17.6%	24.2%	-25.5%
TE/TM=180/40ms	N/A	N/A	N/A	N/A	N/A	N/A
Zero Quantum Filter						
TE1/TE2/TE3=50/9/30ms (maximum ml signal)				8.5%	8.9%	~9%
TE1/TE2/TE3=75/9/30ms (optimized ml/background ratio)				N/A	N/A	N/A
Single Quantum Filter						
SQF, TE=90ms				6.9%	7.5%	~10%

Table 2

Results for simulations performed at double the clinical SNR level. The percent coefficient of variation (%CV), the Cramer Rao lower bounds (CRLB's), and absolute error (expressed as a percentage) are presented for both the ml and (ml+Gly) measurements for the ten pulse sequences considered

Pulse Sequence	% CV (ml+Gly)	CRLB's [%]	absolute error (ml+Gly) [%]	% CV ml	CRLB's [%]	absolute error ml [%]
PRESS						
TE=35ms	2.6%	3.9%	1.0%	6.8%	9.5%	-6.5%
TE=15ms	2.2%	3.1%	-3.7%	4.1%	6.4%	-2.1%
Car Purcell echo train						
CPRESS2 (TE=45ms)	1.9%	3.0%	1.8%	4.6%	6.9%	6.1%
CPRESS4 (TE=67ms)	1.9%	2.6%	4.4%	2.7%	4.0%	13.7%
CPRESS6 (TE=89ms)	2.3%	3.0%	3.8%	3.2%	4.5%	13.1%
STEAM						
TE/TM=5/5ms	4.4%	5.1%	-0.6%	8.8%	12.1%	-17.1%
TE/TM=180/40ms	26.8%	31.0%	-37.0%	N/A	N/A	N/A
Zero Quantum Filter						
TE1/TE2/TE3=50/9/30ms (maximum ml signal)				4.4%	5.0%	~10%
TE1/TE2/TE3=75/9/30ms (optimized ml/background ratio)				11.5%	12.1%	~15%
Single Quantum Filter						
SQ, TE=90ms				3.6%	4.0%	~10%