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Efficient spectroscopic imaging by an optimized encoding of pre-targeted resonances

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Abstract

A “relaxation-enhanced” (RE) selective-excitation approach to acquire *in vivo* localized spectra with flat baselines and very good signal-to-noise ratios –particularly at high fields– has been recently proposed. As RE MRS targets a subset of *a priori* known resonances, new possibilities arise to acquire spectroscopic imaging data in a faster, more efficient manner. Hereby we present one such opportunity based on what we denominate Relaxation-Enhanced Chemical-shift-Encoded Spectroscopically-Separated (RECESS) imaging. RECESS delivers spectral/spatial correlations of various metabolites, by collecting a gradient echo train whose timing is defined by the chemical shifts of the various selectively excited resonances to be disentangled. Different sites thus impart distinct, coherent phase modulations on the images; condition number considerations allow one to disentangle these contributions of the various sites by a simple matrix inversion. The efficiency of the ensuing spectral/spatial correlation method is high enough to enable the examination of additional spatial axes via their phase encoding in CPMG-like spin-echo trains. The ensuing single-shot 1D spectral / 2D spatial RECESS method thus accelerates the acquisition of quality MRSI data by factors that, depending on the sensitivity, range between 2 and 50. This is illustrated with a number of phantom, of *ex vivo* and of *in vivo* acquisitions.

Keywords

MRSI; Relaxation-enhanced acquisitions; brain metabolic imaging; fast spectroscopic imaging

1 Introduction

Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) play crucial roles in deciphering the intricate relationships between the brain’s structure, function, and metabolism [1,2]. MRI probes structural aspects of the brain from a multitude of contrast mechanisms that portray different magnetic properties of water, whereas MRS can probe metabolites residing in the investigated tissues and discern among them using the

fingerprints imparted by their individual chemical shifts. By focusing on molecules that control the bioenergetics, neurotransmission, osmolyte content and other basic properties of the brain, MRS can in principle reflect a complementary –and perhaps more detailed– picture about the nature of healthy function as well as diseased states, than that afforded by the single, ubiquitous water peak. Targeting these resonances, however, can be a challenging task that becomes even more arduous if the spectroscopic measurements are to be combined with their spatial location mapping, to yield a high-dimensional Magnetic Resonance Spectroscopic Imaging (MRSI) view of the brain [1–3]. MRSI's challenges arise from a number of mutually reinforcing factors. These include (i) the fact that resolving a spectrum along the chemical shift dimension requires measuring the signal in the absence of an imaging gradient, via the acquisition of a fairly long (~100 ms) free induction decay (FID); (ii) the fact that such gradient-free acquisition imposes a need to impart the spatial encoding using nested, indirectly-detected phase-encoded dimensions further prolonging the measurement time; (iii) the fact that metabolites are usually characterized by T_1 values that may limit the repetition rates of the experiments; (iv) the fact that observing a ^1H spectrum requires actively suppressing the ca. 10^4 x more intense water resonance falling in the center of the MRS trace, a process which if imperfect leads to baseline distortions and complicates the retrieval of the metabolic images. Despite the enormous potential of spatially resolved metabolic spectroscopy for correlating a brain's structure and its metabolism, the above-mentioned factors render MRSI a challenging technique.

The perturbing presence of the water resonance on the MRS peaks and spectral baseline, demands the use of efficient methods for avoiding its influence. Traditionally these have included water suppression sequences, whereby the water signal is purposely dephased and/or nulled using selective pulses, interspersed with proper field gradients and adequate delays [4–6]. Alternative approaches avoid this and excite water together with the targeted metabolites, relying on the large dynamic range of contemporary NMR receiving systems to avoid saturation [7–9]. Recent studies have also demonstrated the convenience of carrying out ^1H MRS using highly selective pulses, targeting only the resonance frequencies of *a priori* known resonances of interest [10,11]. Driving the use of these selective excitations is the fact that although numerous metabolites can in principle be resolved in brain [3], most studies focus on a number of prominent resonances associated to specific, usually well-resolved signals. It has been shown for instance that so-called “relaxation-enhanced” (RE) methods that carefully craft selective excitation pulses targeting the prominent resonances of Creatine (Cre), Choline (Cho), N-Acetyl-Aspartate (NAA) and Lactic acid (Lac) under ultrahigh-field operation, could enable the acquisition of *in vivo* localized spectra with flat baselines and excellent signal-to-noise ratios (SNR) in a single scan [12,13]. The ca. 10-fold sensitivity enhancement noticed for this experiment vis-à-vis comparisons done at lower fields using conventional water-suppressed-based MRS –for instance on cryoprobe-equipped 9.4 T systems using CHESS-based methods– benefits from an enhanced longitudinal relaxation arising from a proton bulk reservoir that is left mostly unperturbed by the carefully crafted selective excitation pulses, and of very high fields biasing the longitudinal and transverse relaxation properties to the benefit of the metabolic resonances over the water counterpart.

With this RE MRS approach as background, the question arises whether these high sensitivity experiments could be endowed with imaging dimensions. The sensitivity penalties associated to MRSI are much higher than those of localized MRS, given the former's need to spread out the spectral peak integral over a large number of voxels –with a concomitant decrease in each voxel's peak intensity. On the other hand, the fact that RE MRS transforms an in-principle continuous spectral acquisition into a sparse, binned one targeting a small number of resonances whose positions are *a priori* known, opens new possibilities to acquire this MRSI information in a faster, more efficient manner. Indeed, with evolution frequencies known, the images of the various targeted metabolites can in principle be resolved by collecting a series of conventional imaging data, where the chemical shifts of the different resonances have been differentially encoded as coherent phase modulations amenable to disentanglement by post-processing. Such chemical shift encoded techniques have been demonstrated in the past, using either phase-modulated pulses or suitable delay times imparted in different scans [14–16]. The present study describes the principles, the implementation and the potential of alternative MRSI forms based on imparting the modulations in unison with the relaxation-enhanced excitation and with gradient echo trains, in what we denote as Relaxation-Enhanced Chemical shift-Encoded Spectroscopically-Separated (RECESS) MRI. As shown, the sparse nature of the resulting acquisition scheme may lead to significant advantages in terms of scanning time and SNR.

2 Basis of RECESS MRI

The starting point of this study is the RE MRS sequence shown in Fig. 1a [10]. The sequence begins with a 90° pulse designed to excite solely *M* bands containing the metabolites of interest in the MRS study. This pulse was here built based on the Shinnar-Le Roux (SLR) algorithm [17], which in addition to exciting the desired bands with a linear-phase equiripple, avoids the water resonance peak at ca. 4.80 ppm (with peak excitation amplitude at $\approx 10^{-4}$). As such selectivity ends up requiring a relatively long pulse the experiment is executed in a spin-echo format, incorporating a 180° adiabatic refocusing pulse based on a Sech/Tanh modulation [18,19] covering solely the excited bands. To complement the spectral selectivity of these pulses with spatial selectivity, the original RE MRS sequence also incorporates three pairs of LASER pulses [20] that circumscribe the observed region to a rectangular/cubic voxel in space.

On considering how to extend this localized MRS scheme towards spectroscopic mapping, the simplest option is to complement the LASER localization by a phase-encoding of the spatial dimensions, as is done in conventional chemical shift imaging (CSI) [21,22]. Figure 1b illustrates the resulting experiment, assuming encoding along two spatial axes and LASER-based slice selection along the third one. While enjoying potential benefits from relaxation enhancement, a drawback of this CSI scheme is its long intrinsic duration. This can be dealt with by noticing a redundancy in this particular experiment, which assumes *a priori* known peak positions for performing the excitation, but at the same time devotes a full time-domain FID to characterize the same frequency peaks. One way to solve this is by introducing a “signature” which will allow one to resolve the image corresponding to each peak, without demanding a full Fourier analysis of the FID. This can be implemented by phase-modulating each resonance, either by the addition of suitable phase factors upon

implementing the spectral excitation pulse [23,24], or by introducing an array of suitably timed delays. Figure 1c illustrates the latter implementation, that builds on the RE CSI sequence in Fig. 1b, but replaces one of the phase-encoded loops by a combination of shift-encoding time delays and of spatial-encoding gradient echoes. RE MRS's excitation and refocusing of the $M\{\Omega_m\}_{1 \leq m \leq M}$ targeted resonances thus remain unchanged, and so does the slice selection carried out by the 1D LASER block. The acquisition, however, is now replaced by a train of N readout gradients ($N \gg M$) incorporating gradient echoes at a set of suitable intervals $\{\tau_n\}_{1 \leq n \leq N}$. Each of these gradient echoes will provide a full imaging set, with the contribution of each different site modulated by individual chemical shift factors $e^{i(\Omega_m \tau_n)}$. Assuming that each image acquisition segment is sufficiently short to disregard intra-segment modulations among the individual sites (*i.e.*, $|\Omega_k - \Omega_j| \tau_n \ll 1 \forall k, j$) and given that each image acquisition is followed by a full gradient echo, it is possible to describe the n -th observed echo signal as:

$$S_n(k, \Omega_m) = \sum_{m=1}^M \int \rho(r, \Omega_m) \cdot e^{ikr} e^{i(\Omega_m \tau_n)} dr, \quad n=1, 2, \dots, N \quad (1)$$

FT of each of these k -space signals then yields a set of N images

$$I_n(r, \Omega_m) = \sum_{m=1}^M \rho(r, \Omega_m) \cdot e^{i(\Omega_m \tau_n)}, \quad n=1, 2, \dots, N \quad (2)$$

Notice that each of these images mixes contributions from all the spectral counterparts, with phases modulated by an encoding $E_{mn} = \{e^{i(\Omega_m \tau_n)}\}$ phase-factor matrix. Rather than choosing this encoding matrix according to Nyquist, fast FT criteria, we choose the time intervals $\{\tau_n\}_{1 \leq n \leq N}$ so as to endow E_{mn} with a low condition number –and hence with the possibility of performing a stable inversion of Eq. (2) so as to separate the various $\rho(r, \Omega_m)$ contributions. Many routes could be devised for finding such optimum set of time intervals for a given subset of resonances of interest. In the present study a simple approach was adopted, whereby all the encoding delays were assumed to be multiples of a basic interval τ : $\tau_n = n\tau$, $n = 1, 2, \dots, N$. A convenient feature deriving from this choice is that searching for a stable inversion of the chemical shift encoding matrix E becomes a straightforward search for the matrix's minimum condition number –for instance, using Matlab's® (The MathWorks, Natick, MA) “*cond(E)*” script– as a function of only two parameters: τ and N . A generalized inversion of a suitably conditioned E then yields a stable decoding transformation matrix D_{mn} capable of delivering “pure” images for individually separated resonance as:

$$\rho(r, \Omega_m) = \sum_{n=1}^N D_{m,n} \cdot I_n(r, \Omega_m). \quad (3)$$

A further improvement associated with this shift-encoding mode, relates to the fact that the overall time $T_{acq} = N\tau$ required to suitably encode a small number of resonances is typically short; well within the acquisition boundaries placed by typical metabolic T_2 s. This opens up the possibility of further extending the 1D phase-encoded gradient-echo scheme in Fig. 1c to a single-shot sequence involving both gradient- and spin-echoes [25,26], as shown in Fig. 1d. In this sequence the previously multi-shot dimension is now probed in a single shot thanks to a Carr-Purcell Meiboom-Gill (CPMG) scheme [27,28] incorporating the phase encoded information as a series of N_{pe} blips. The addition of 180° refocusing pulses –kept selective and circumscribed to an even number to ensure that the bulk reservoir of untouched magnetization remains unperturbed– brings about periodic reversals of the chemical shift evolution, which amount to switching between $\{E_{mn}\}$ and $\{E_{mn}^*\}$ chemical shift encoding matrices for each phase encoding step. These refocusing pulses necessitate as well a reversal in the signs of the readout gradients between even and odd spin-echoes, to maintain a steady sampling of the signal near the k -origin. Moreover, when considering the arrangement of the chemical shift encoding elements in the generalized (k_x, k_y, τ_n) 3D space where the spin evolution associated to this experiment takes place, the shift-related evolution advances in a “positive” way in the first N readout echoes, and reverses its progress in the next subsequent N echoes. In other words a modulation $\{E_{mn}\}$ drives the chemical shift encoding before the odd 180° pulses, whereas its complex conjugate $\{E_{mn}^*\}$ defines the shift encoding before the even ones. Figure 2 illustrates this feature, assuming that the readout and phase encodings are associated to x and y axes, respectively. Based on this notation one can extend the shift-encoding expression in Eq. (1) to:

$$S_n(k_x, k_y, \Omega_m) = \sum_{m=1}^M \rho(x, y, \Omega_m) \cdot e^{ik_x x} e^{ik_y y} e^{i(\Omega_m n \tau)} dx dy. \quad (4)$$

Arguments akin to those leading to Eq. (3) but relying on a 2D FT with respect to k_x, k_y , lead to a series of “pure” images for each of the addressed resonances,

$$\{\rho(x, y, \Omega_m)\}_{m=1,2,\dots,M}.$$

In summary, it follows from these considerations that execution of the single-shot experiment in Fig. 1d requires

1. Setting up a 1D RE-based MRS acquisition incorporating selective pulses, exciting a set of *a priori* known resonances of interest $\{\Omega_m\}_{1 \leq m \leq M}$ according to the scheme in Fig. 1a.
2. On the basis of this reference spectrum, find a suitable number $N (N \geq M)$ of τ intervals that endow a low condition number to an encoding matrix $\{E_{mn}\}_{1 \leq m \leq M, 1 \leq n \leq N}$. If more than one such solution exists, the shortest possible combination compatible with gradient switching times is chosen to minimize T_2 -derived losses.
3. With these parameters, the RECESS sequence in Fig. 1d can be set up and executed. Still, to be able to jointly co-process along the k_x -axis the even/odd

data sets arising from the E/E^* encoding matrices, an additional set of reference scans lacking the phase encoding blips may be needed. This procedure is akin to the need to collect a reference scan for correcting artifacts in the joint processing of even and odd readout echoes in echo planar imaging [29]. With this additional acquisitions performed, a suitable phase correction is found that enables, by multiplication of each readout data set in the CPMG train, to merge the $2N$ chemical-shift encoded sets of continuously sampled data, into mutually compatible 2D k -space data.

4. With data corrected in such manner, the generalized inversion matrix of $E_{mn} = \left\{ e^{i(\Omega_m n \tau)} \right\}$ is employed to decode, out of the N k -spaces probed with positive gradient readout echoes, the corresponding M chemical shift decoded k -space signals. Likewise, the generalized inversion matrix of $E_{mn}^* = \left\{ e^{-i(\Omega_m n \tau)} \right\}$, is used to decode the M shift-resolved k -space data sets that were collected using negative gradient readout echoes.
5. These two shift-decoded data sets can now be re-interleaved to provide fully sampled readout and phase-encoded k -spaces, resolved for each $\{\Omega_m\}_{1 \leq m \leq M}$ frequency.
6. 2D fast FT of these k -domain data provides final, separate images for each of the targeted chemical shifts.

3 Experimental

The performance and robustness of these approaches were assayed on a phantom composed of four components (water, methanol, acetone, cyclohexane), on a fresh *ex-vivo* mouse brain sample targeting the NAA, tCho, tCre and a residual water resonance, and on a mouse's abdomen for the sake of exploring water/fat separation. These acquisitions were carried out on a 7/120 T horizontal magnet MRI using a quadrature birdcage coil probe (Agilent Technologies, Santa Clara, CA). As an aid in setting up the sequences in Fig. 1, spin-echo multi-shot (SEMS) imaging and point-resolved (PRESS) localized spectroscopy experiments were also carried out, using sequences taken from the scanner's software libraries. To better assess the performance of the new multi- and single-shot RECESS sequences, these were also compared against the more conventional multiscan CSI approach shown in Fig. 1b. The radiofrequency (RF) pulses in all these sequences were designed on the basis of the SLR algorithm [17], computed using a Matlab[®] script, and uploaded onto the scanner for execution. Optimization of the number and duration of the encoding delays based on the conditioning of the chemical shift encoding matrix $\{E_{mn}\}$ (possessing an identical inversion stability as $\{E_{mn}^*\}$), were also carried out using Matlab scripts. All the data rearrangements, phase corrections and shift decoding procedures required by the RECESS experiments were written as embedded macros in the scanner's original VNMRJ[®] programming environments; this provided the possibility of executing the image/spectral processing involved directly upon acquisition environment, and enjoy from native Fourier processing, phase correction and image display capabilities. All of these Matlab and VNMRJ preparation and processing scripts and macros, are available upon request. Animal protocols and maintenance were

done in accordance with guidelines of the Institutional Committee on Animals of the Weizmann Institute of Science (IACUC protocol 10790514).

4 Results

Figure 3 exemplifies how the $\{\tau_n = n \cdot \tau\}_{1 \leq n \leq N}$ arrays defining the chemical shift encoding matrices required for RECESS's spectral resolution, were chosen for different sets of pre-defined chemical shifts. The Figure's left-hand panel shows the changes in the encoding's matrix E condition number as a function of the two parameters (N and τ) involved. These calculations assumed an excitation addressing solely three ^1H resonances in a Methanol/Acetone/Cyclohexane phantom, resonating at 4.03, 2.99 and 1.86 ppm respectively. As follows from these graphs a suitable number of gradient echoes per phase encoding step arises when $N = 6$ and $\tau = 1.002$ ms (Fig. 3a); the ensuing condition number is sufficiently low to ensure a stable matrix inversion, while the encoding duration is sufficiently short to enable the implementation of CPMG acquisitions. The center panel of Fig. 3 shows an extension of this test to address the *in vivo* methyl peaks of Cho, Cre and NAA, resonating at 3.09, 2.93 and 1.92 ppm respectively. A fourth frequency peak at 4.80 ppm was also incorporated, corresponding to the resonance that may arise in *in vivo* spectroscopy if water suppression is incomplete. Under these conditions, $N = 8$ gradient echoes lead to a good condition number 1.843 at $\tau = 1.524$ ms (Fig. 3b); although a $N = 10$ train would lead to a lower condition number, the overall encoding time ($\approx 10 \times 1.62$ ms) would be too long to justify the ensuing gain. Another aspect analyzed in Fig. 3c, is how disturbances in the presumed chemical shifts will affect the method's site separation abilities. It can be readily shown that if the changes originate from relatively small field heterogeneities, so that at a single-voxel level all the peaks to be resolved move in unison, the condition number of the encoding matrix remains unchanged. If field heterogeneities become so large that even within a given voxel the peaks exhibit a relative fluctuation with respect to one another, the condition number changes. As evidenced by Fig. 3c these random changes will have only minor effects in the inversion properties of the encoding matrix, hence evidencing the method's robustness *vis-à-vis* larger field heterogeneities. Overall, these examples demonstrate that low condition numbers can usually be found for sparse spectral scenarios like the ones addressed by RE MRS, leading to a short and efficient chemical shift encoding that is robust against common field distortions while enabling the execution of CPMG spin-echo acquisitions.

Figure 4 compares the relative signal-to-noise ratios (SNRs) as well as residual cross-talk levels among the different locations of various chemical sites, arising upon comparing RECESS and optimized relaxation-enhanced CSI experiments. These tests examined a phantom consisting of an outer 25 mm water tube, and three inner 10 mm tubes with methanol, acetone and cyclohexane (labeled as I, II, III respectively; Fig. 4a). These tests were repeated as a function of a global field inhomogeneity (Figs 4b-4d). As can be seen from Figs. 4e-4i, all sequences succeed in separating the three different chemical shift components that were targeted, and in providing quality 2D images for each. This validates the chemical shift encoding modules of the RECESS sequences, which have no particular difficulty in separating the various sites *vis-à-vis* conventional phase-encoded chemical shift

imaging—even under the presence of moderately inhomogeneous fields. This separation ability is further reinforced by the similar levels of cross-talk artifacts that under similar field homogeneity conditions, are evidenced by the 2D phase-encoded CSI results (Fig. 4e) as for its RECESS-based counterparts (Figs. 4f, 4h). As expected from Fig. 3c, these cross-talk levels do not worsen to a statistically-significant degree after degrading the field inhomogeneity (Figs. 4g, 4i). At the spatial resolution level the performance of the various sequences is harder to assess, since for similar Fields-of-View (FOVs) and matrix sizes as those achieved by a RECESS single shot acquisition (which employed a 5 sec pre-acquisition delay TR plus another 5 sec for recording a reference navigator set), a RE CSI sequence would require ~6 hours of continuous acquisition. Hence, for assessing the SNR of each chemically-resolved imaging technique—as measured as the average of a targeted chemical component divided by the standard deviation of a noise-only region—the pixel size of the CSI reference was taken four times as large as those of their RECESS counterparts. The ensuing results are summarized in the various panels of Figure 4. Remarkably, even if factoring in these different pixel sizes, the ensuing SNRs are not overtly different for each of the recorded sets. The SNR of the multi-scan phase-encoded RECESS version (Fig. 4f) is on average a factor of two lower than that of the lower-resolution CSI counterpart (Fig. 4e)—yet at the expense of the latter's thirty-fold longer acquisition times. This is a reflection of the much more efficient encoding that RECESS sequences make of the *a priori* targeted chemical shift information. Another factor of two separates the multi-shot from the single-shot (Fig. 4h) RECESS versions—yet once again at a saving of nearly thirty-fold in the duration of the experiment. This is now probably reflecting the efficient use that the CPMG pulse train in the latter sequence makes of the short acquisition time in τ -encoded RECESS sequences, and of the sensitivity gains that are to be made by echoing what are usually inhomogeneously broadened—but not necessarily short- T_2 —spectral resonances.

Figure 5 shows extensions of these phantom tests to *ex-vivo* mouse brain acquisitions. A comparison between PRESS (Fig. 5b) and RE spectra (Fig. 5c) demonstrates an average SNR enhancement per unit time of ca. 25%, when the latter focuses on three peaks reflecting total Cholines, total Creatines and NAA. Also noticeable is a nearly complete absence of the water peak in the RE spectrum. Figures 5d-5f illustrate the metabolic maps afforded by the “single-shot” RECESS sequence for tCho, tCre and NAA, while Figs. 5g-5i present counterparts arising from a RE phase-encoded CSI sequence as in Fig. 1b. While both of these sequences benefit from a potential longitudinal relaxation enhancement, the first of these sets displays a slightly superior sensitivity despite requiring ca. 50% of the latter's acquisition time. This might be a consequence of its reliance on a CPMG train, capable of overcoming potential T_2^* losses.

Finally, Figure 6 demonstrates an application of these strategies to the spectroscopic imaging of fat and water peaks resonating at 4.8 and 1.5 ppm respectively, in a live mouse. Because of the good sensitivity of this abdominal experiment, the possibility arose of exploiting the single-shot nature of the RECESS sequence introduced in Fig. 1d. Limiting this potential, however, were the relatively short T_2 s of the targeted species. Analysis of this system indicated that at 7T a stable separation of the two sites would result from $N=3$ echoes separated by $\tau = 0.668$ ms. If these parameters were inserted in the multi-scan phase encoded sequence illustrated in Fig. 1c, the result would be a three-point Dixon-like

water/fat separation sequence [14]. Figures 6b and 6d illustrate the retrieval of such images in a single scan (plus a reference one); the correspondence with images collected in multiple shots in the presence of fat or water suppression (Figs. 6a, 6c), is evident.

5 Discussion and Conclusions

The encoding modes employed by the RECESS sequences here illustrated leverage a number of complementary aspects, deriving from the *a priori* knowledge of the resonance positions assumed by the RE MRS experiment. These include the sparse nature of these resonances, which makes their disentanglement via a short number of encoding delays associated to a stable numerical inversion feasible; the possibility of concatenating these encoding delays in a train of gradient echoes, adding a spatial dimension to the spectral decoding procedure while retaining the experiment's single-scan nature; and the shortness of even this gradient echo train, enabling its concatenation within a CPMG loop capable of phase-encoding an additional spatial axis. This leads to a significant compression of what eventually becomes a 2D spatial / 1D spectral single-shot acquisition. On a per-scan basis the sensitivity of the new resulting method appeared to be ca. twice as high as that resulting from more conventional, phase-encoded CSI counterparts; this is likely the result of the additional sensitivity endowed by the spin-echoed acquisition train vis-à-vis a regular FID acquisition. Interestingly, when applied on a two-site system, the ensuing sequence becomes a multiple gradient- and spin-echoed version of the Dixon water-fat separation experiment – which is the archetypical *in vivo* scenario where the targeted frequencies are *a priori* known.

Notwithstanding these benefits, a number of factors may challenge RECESS's effectiveness. Foremost among these are any issues that compromise the experiment's sparse spectral assumption. Indeed, if executed at low magnetic fields, if attempting to separate too many resonances, or if investigating systems that are spectrally crowded, the excitation pulses may become too long and/or decoding gradients require too high a number N of echoes. T_2 -derived losses may in any of these cases become too onerous to make the approach competitive. Less important are field inhomogeneity and chemical shift dispersion effects, for which the condition number of the chemical shift encoding matrix can apparently often be optimized without increasing the number of gradient echoes. A final aspect that conspires against an optimal operation of the RECESS approach, arises from the need of collecting reference scans to enable a suitable co-processing of the phase-encoded k -space. While reference scans are easily incorporated into sensitive EPI acquisitions, they can significantly increase experimental times in low sensitivity MRSI investigations. It is to be hoped that either perfection of the imaging hardware and/or referenceless methods developed to deal with phase corrections in EPI-based experiments [31,32], may also be useful in the present approach. This would be of particular value not only on low-sensitivity ^1H MRSI experiments like those illustrated in this study, but also in higher-sensitivity but less reproducible measurements like those involved in hyperpolarized ^{13}C MRSI.

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Abbreviations

CPMG	Carr-Purcell Meiboom-Gill
CSI	Chemical Shift Imaging
EPI	Echo-Planar Imaging
EPSI	Echo-Planar Spectroscopic Imaging
FID	Free Induction Decay
FOV	Field of View
FT	Fourier Transform
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MRSI	Magnetic Resonance Spectroscopic Imaging
PE	Phase-Encode
RE	Relaxation-enhanced
RECESS	Relaxation-Enhanced Chemical-shift-Encoded Spectroscopically-Separated
RF	Radio frequency
RO	Read-Out
SNR	Signal-to-Noise Ratio
TE	Echo Time
TR	Repetition Time

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Figure 1.

Principles of RECESS MRSI. (a) Basic RE MRS sequence [10,13] incorporating a 90° SLR multiband selective pulse, a series of 180° pulses providing refocusing and 3D LASER localization, and a FID acquisition. (b) In a chemical shift imaging (CSI) extension, this localized spectroscopy is endowed with 2D spatial resolution by the addition of two phase-encoding gradients. (c) In RECESS MRSI the spectroscopic information is no longer retrieved by fast Fourier Transform (FT) of a FID, but via N refocused readout gradients whose echoes are timed at intervals τ that enable –following a FT of the echoes along their k -evolution axes– a stable matrix-based inversion delivering each of the metabolites' images. A τ_1 delay is introduced to refocus the gradient-imposed evolution without disturbing these shift-encoding steps. (d) Given the short duration of the RECESS chemical-shift spectral decoding in (c), this scheme can be extended by a CPMG-based echo train that carries out a phase encoding of the remaining spatial dimensions (N_{pe} loops) in a single scan. In (b), (c) and (d), the spatial encoding of the MRS information is assumed along only two axes, leaving a need for a 1D LASER block to localize the remaining dimension.

Figure 2.

Transverse of the (k_{ro}, k_{pe}, τ_n) space in the single-shot RECESS acquisition protocol, highlighting details of the chemical shift encoding and decoding modes. (a) Expanded acquisition block of the sequence, showing only the first and last acquisition gradient echoes for each phase encoding step. (b) Trajectories imposed on the spins' evolution along the chemical shift encoding, the k_{ro} and the k_{pe} imaging dimensions, highlighting the positions of ten instants marked in the sequence (a). Green arrows represent k_{ro} -axis traces scanned during six hypothetical readout gradients for each chemical-shift-encoding train; the dotted green lines next to them represent the trajectories imposed by the corresponding refocusing gradients. The solid cyan lines show the jumps along k_{pe} imposed by the phase encoding gradient blips and by the refocusing pulses; notice in this case the alternating changes in k_{pe} values. In each phase encoding step the signals collected in the presence of positive readout gradients ($k_{pe} = 0$; darker green colors) are modulated by $\{E_{mn}\}$, while those recorded during negative readout gradients ($k_{pe} > 0$; lighter green) are modulated by $\{E_{mn}^*\}$. (c) Details of the chemical shift decoding procedure. The $N \times N_{pe}$ full k_{ro} -space echoes carrying the chemical shift encoded information, are separated into two matrices according to the sign of the readout gradient employed. For each phase-encoded value, the N echoes recorded in the presence of positive readout gradients are decoded by the inverse of the encoding matrix $\{E_{mn}\}$, while shift-encoded signals recorded while in the presence of negative readout gradients are decoded by the inverse of $\{E_{mn}^*\}$. Each of the ensuing 2D k -space data sets is then fast FT for retrieving what in principle are identical spectrally-resolved images (procedure not shown); these can be co-added for the sake of improving sensitivity.

Figure 3.

Optimizing the condition number of a chemical shift encoding matrix $E_{mn} = \{e^{i\Omega_m n \tau}\}$, assuming $\tau_n = n\tau$, $n = 1, \dots, N$. (a) Phantom sample scenario involving the excitation of three chemical sites at 4.03, 2.99 and 1.86 ppm, showing how condition numbers change as a function of τ and N . Although equally good encodings appear for a variety of combinations, an encoding based on $N=6$ gradient echoes equispaced by $\tau = 1.002$ ms provides both low condition number (1.08, red) and timings that can be accommodated by the gradient echo train without being overly short or long. (b) Idem but for a hypothetical mouse brain sample, involving the excitation of residual water plus Cho, Cre and NAA methyl peaks at 4.80, 3.09, 2.93 and 1.92 ppm. A suitable condition number = 1.84 (red) is then reached for $N=8$ and $\tau = 1.52$ ms; although a better conditioning is achieved by $N=10$, the increased number of gradient echoes coupled to the longer $\tau = 1.62$ ms intervals would lead to unnecessary T_2 -derived losses. (c) Examining the robustness of E 's conditioning, with ten cases where the frequencies of the four resonances targeted in (b) were varied randomly over 20 Hz, for N fixed at 8 gradient echoes.

Figure 4.

Comparing the spectroscopic imaging results from a phantom sample involving water, methanol (I), acetone (II) and cyclohexane (III), arising from various versions of relaxation-enhanced sequences. (a) Reference spin-echo multi-shot image. (b) Reference spectrum acquired by PRESS [30], delivering the spectral information required to design the SLR pulse (the small unlabeled resonance at ~5.5 ppm corresponding to the untargeted -OH methanol site). (c,d) Spectra acquired using the spatially-localized RE MRS sequence in Fig. 1a, in well shimmed and in poorly shimmed magnetic fields, respectively. A 40 ms multiband linear-phase equiripple pulse was used to excite the (I, II and III) resonances; the line widths of resonance III were 12 Hz in (c) and 75 Hz in (d). (e) Spectrally resolved images of the three components arising from a Relaxation-Enhanced but otherwise conventionally phase-encoded CSI sequence like the one introduced in Fig. 1b, collected in the well-shimmed field. (f,g) Spectrally resolved images of the three targeted components arising from the multi-scan shift-encoded RECESS sequence introduced in Fig. 1c, collected in a well-shimmed and in an inhomogeneous field, respectively. (h,i) Idem but for the single-scan multi-echo RECESS MRSI sequence shown in Fig. 1d. Regions of interests for SNR and for residual cross-talk level calculations were marked with squares in (a). The SNR for each image was measured by dividing the average of the corresponding component signals within the marked green/magenta/blue squares by the standard deviation of the noise in the red-marked square. The same markers were also used to calculate residual levels in panels (e)-(i), by dividing the targeted signal by the residuals arising at the positions of the remaining two components. Common scan parameters: FOV = 40 × 40 mm², slice thickness = 2 mm, two dummy scans, echo time = 50 ms, TR = 5 s, averages nt = 1. For the RECESS experiments, the shift-encoding parameters were $\tau = 1.002$ ms, $N = 6$. The matrix size for

(e) was 64×64 and its total scan time is 5 h 41 min. Matrix sizes for (f), (g), (h) and (i) were 128×128 ; total scan times for (f) and (g) were 10 min 40 sec, while scan times for (h) and (i) were only 20 sec.

Figure 5.

Comparison of *ex-vivo* mouse brain results arising from RECESS and from a RE-based CSI sequences. (a) Reference SEMS image. (b) Reference PRESS spectrum acquired on a $16 \times 16 \times 4 \text{ mm}^3$ voxel using 32 averages. (c) RE MRS spectrum acquired on the same voxel and scan numbers as (b), but using the sequence in Fig. 1a with a 40 ms two-band pulse exciting the total Cholines (tCho) and total Creatines (tCre) in one band, and N-Acetyl Aspartate (NAA) in another. (d-f) Cho, Cr, NAA maps (overlaid on anatomical T_1 -weighted images) arising from the execution of the CPMG-based RECESS sequence in Fig. 1d. (g-i) Idem but

for acquisitions based on a RE CSI sequence incorporating independent phase-encoding loops. Common scan parameters: FOV = $16 \times 16 \text{ mm}^2$, slice thickness = 4 mm, matrix size = 32×32 , echo time = 50 ms, TR = 2 s. RECESS parameters: $\tau = 1.524 \text{ ms}$, $N = 8$, number of averages = 2048. The number of averages for the RE CSI sequence was 8 per phase-encoding value. The experimental time of the RECESS MRSI experiment was 2 h 16 m (including the reference scans) while the phase-encoded CSI acquisition took 4 h 33 m.

Figure 6.

In vivo fat/water separation capabilities of the RECESS sequence in Fig. 1d, as applied to abdominal mouse investigations at 7T. (a, c) Multiscan spin echo references involving fat suppression (a) and water suppression (c). (b, d) Water- (b) and fat-tissue (d) images separated by single-scan RECESS acquisition. Common imaging parameters: FOV = 32×32 mm², TR = 2 sec, TE = 14 ms, slice thickness = 2 mm. The reference image matrix size was 128×128; due to the short T₂s the RECESS image size was 64×64, which explains the lower

resolution. Total scan time for each spin-echo multi-scan image: 4 min 16 sec. Total RECESS acquisition time (including a reference navigator scan): 4 sec.