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Review

# Measurement sequences for single voxel proton MR spectroscopy

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**Abstract**

Proton spectroscopy is easy to perform at clinically used whole body MR scanners, since the necessary hardware is identical to that of MR imaging. Only specific measurements sequences for the acquisition of volume selected spectral information and appropriate evaluation software have to be installed. Several techniques for a volume-selective spectroscopy have been proposed, but only two are widely used in clinical examinations: the double spin-echo sequence (point resolved spectroscopy sequence (PRESS)) and the stimulated echo sequence (stimulated echo acquisition mode (STEAM)). The properties of these sequences are described and additional techniques for artifact reduction and the extraction of selected signals are explained.


*Keywords:* Volume selection; Double spin-echo; Stimulated echo; Metabolite nulling

## Contents



## 1. Introduction

Human proton spectroscopy can be performed with all conventional MR scanners having field strengths of 1.5 T or higher. No additional hardware is necessary to perform spectroscopic measurements at a MR scanner, only special measurement sequences and evaluation procedures have to be installed. In this paper, the hardware components necessary for proton spectroscopy are shortly described and the mainly used measurement sequences are explained.

Proton spectroscopy of fluids was performed very early after the invention of the principles of nuclear magnetic resonance. As an example, the proton spectrum of alcohol was described by Arnold et al. [1] already 1951. Some years later proton spectroscopy was already used for the analysis of unknown substances. Meyer et al. [2] provided a list of chemical shifts of organic compounds which could be used for the identification of unknown substances. These early results were obtained by the so-called sweep experiments. The reaction of the probe was examined successively for different resonance frequencies, which was very time-consuming. An important improvement of spectroscopic measurements was the observation of Ernst and Anderson [3], that the complete spectrum can be analyzed by Fourier transformation of the time signal obtained after a short radio frequency pulse.

For the application of MR spectroscopy in living systems it was necessary to restrict the origin of the measured signal to a selected part of the volume within the MR scanner. The first

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way to do this was the use of surface coils, which received signals only from a limited volume adjacent to the coil [4]. For many in vivo spectroscopy applications however, it should be possible to choose the origin of the signals arbitrarily within the body. This can be obtained by the so-called static field gradient techniques. In one application, the static magnetic field was shaped in a way, that the magnetic field has the homogeneity necessary for spectroscopy only in the center of the magnet [5]. In the same year, the selection of a single point in the volume without disturbing signals was realized by changing gradient field strengths during the measurement—the so-called "sensitive-point" method [6]. Both of the mentioned techniques realize the volume selection only by static or dynamic field gradients and suffered from a bad defined spatial origin of the obtained NMR signals. The spatial selectivity can be improved by using combinations of linear field gradients and band-selective radio frequency [7] pulses, as usually realized in most imaging techniques. An early proposed measurement sequence with this type was the volume-selective excitation (VSE) sequence by Aue et al. [8]. In this sequence both non-selective and slice-selective excitations were used. Another approaches are the saturation of the longitudinal magnetization of nuclei outside of the region of interest [9] or the encoding of selected slices in the image-selected in vivo spectroscopy (ISIS)-sequence [10], which needs several measurements with following additions and subtractions of signals. This makes the ISIS technique sensitive to movement effects. Better results could be obtained by sequences, which did not excite the whole object. Two of such sequences are the point resolved spectroscopy sequence (PRESS)-sequence [11] and the stimulated echo acquisition mode (STEAM)-sequence [12]. In clinically used MR scanners, these both sequences are usually available for volume-selective spectroscopy. They are described in more details in this article.

## 2. Explanation of the topic

### 2.1. Sequences for volume-selective excitation

In modern single voxel proton spectroscopy, the spatial selectivity of the obtained signals is achieved by a combination of slice-selective excitations. Each of these excitations works identically as in MR imaging: a radio frequency pulse with a specific frequency bandwidth is applied while a field gradient is switched on. Thus, the excitation of nuclei is restricted to a selected slice. By modifying the strength of the field gradient and the center of the radio frequency band, the thickness of the slice and the distance of the center of the slice from the center of the magnet can be modified. The orientation of the selected slice can also be chosen freely by an appropriate weighted combination of the three available field gradients within the magnet. Arbitrary tilted slices can be realized in this way. For a selection of a volume instead of a slice, three slice selections with different orientations are necessary. The selected volume (volume of interest, VOI) is then built by the intersection of the three selected slices.

The combination of different slice selections can be realized within a single measurement sequence in a way that only signals from the selected VOI are obtained (single-shot measurement).

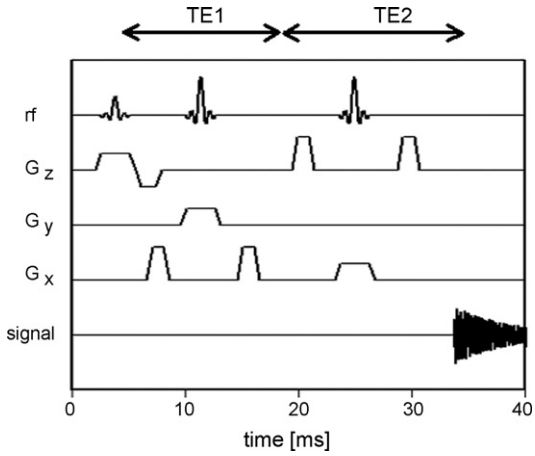


Fig. 1. PRESS-sequence with three rf pulses applied simultaneously with field gradients along the main axes of the magnet. Only the first part of the data acquisition time is shown by the first part of the free induction decay.

In the PRESS-sequence, a 90° excitation and two 180° refocusing pulses are used and in the STEAM-sequence all three pulses are 90° excitation pulses.

The sequence scheme of the PRESS sequence is shown in Fig. 1. In the upper line, the three rf pulses can be seen. Each of them is applied simultaneously with a different field gradient. After the excitation by the first 90° pulse, transversal magnetization is produced within a slice perpendicular to the *z*-axis in this case. This magnetization starts to dephase and after a time TE1/2, the magnetization of a part of the slice is refocused by the first 180° pulse in the same way as in conventional spin-echo sequences. The spin-echo occurring at the time TE1 is not evaluated, the magnetization dephases again and the second 180° pulse is applied at the time TE1 + TE2/2 to give an echo at the time TE1 + TE2, were the data acquisition time starts. In contrast to imaging sequences, where the spin-echo occurs usually in the center of the data acquisition time, the acquisition of data in spectroscopy sequences starts mostly at the center of the spin-echo and lasts for several hundred ms. This is necessary to be able to analyze small frequency differences of few Hertz in the chemical shift of the observed molecules.

The use of the double spin-echo is necessary for the realization of the desired spatial selectivity. The steps of volume excitation are visualized in Fig. 2. The order of used gradients is only an example and can be changed. After selective excitation of a slice in a *xy*-plane by the first 90° pulse, the refocusing effect of the first 180° pulse is restricted to a slice in a *xz*-plane. Therefore, the first spin-echo occurs only for spins within a bar which includes those spatial positions which are part of these both slices. At all other parts of the first excited slice, the magnetization at the echo time TE1 is dephased due to the missing refocusing effect of the 180° pulse. The second 180° pulse affects again a different slice, which is in the *yz*-plane in the example of Fig. 2. Only those spins, which are located in the crossing volume of the former selected bar and the new slice are affected by this refocusing. This crossing volume builds a cube with edge lengths, which correspond to the slice thicknesses of the three rf pulses. Only in this cube a magnetization

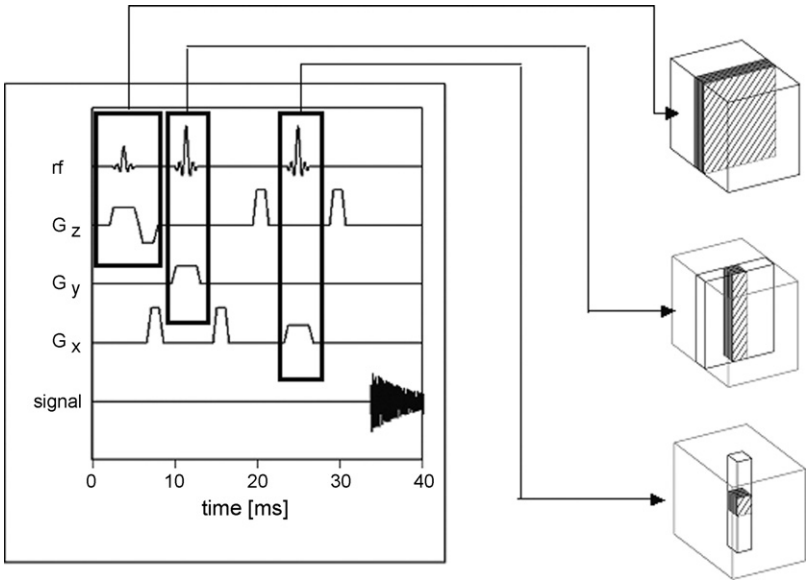


Fig. 2. Selection of a cube with a PRESS sequence. The three rf pulses within the sequence are marked and the selected regions after each pulse are shown for an cubic object.

excited by the 90° pulse and fully rephased by both 180° pulses exists.

The restriction of the acquired signal to this selected volume of interest presumes, however, that all signals from other parts of the examined subject are cancelled out. Usually, the homogeneity of the magnetic field in an examined region of a body is very high and therefore the dephasing due to field inhomogeneities of the static field within the patient is not strong enough to destroy the visible magnetization of the regions outside of the selected voxel of interest (VOI). Furthermore, the last applied pulse, which is nominally a 180° refocusing pulse, affects partly an excitation of the selected slice, since at the border of the slice a zone of flip angles near 90° exists. The signal from this border zone will contribute to the acquired signal.

To avoid these contributions to the signal from nuclei outside the VOI, additional field gradients (so-called spoiler gradients [13]) are applied within the PRESS sequence (Fig. 3). The effect of these spoiler gradients is vanishing for nuclei, which are affected by all three rf pulses – those within the VOI – but outside the VOI they lead to a dephasing effect of the undesirably excited magnetization.

The shape of the VOI shown in Figs. 2 and 3 is only an example of feasible possibilities. In clinical examinations, the slice thickness of all three rf pulses can be chosen independently and also oblique slices, realized by combining of two or three of the *x*-, *y*- and *z*-gradients, can be selected resulting in a tilted cuboid VOI. In the given example, the three selected slices are perpendicular to each other. This is the most usual situation, but it is also possible to use other angles between the slices, resulting in a parallelepiped as the shape of the VOI. Curved and concave surfaces cannot be realized with this measurement technique. Several modifications of the here described conventional PRESS technique for special demands are described in the literature.

The second frequently used sequence for volume-selective spectroscopy is the STEAM-sequence. This technique uses the effect of a stimulated echo [14] occurring after the application of three successive pulses. The most intensive signal strength of a stimulated echo can be obtained, if all three pulses are 90° pulses (Fig. 4). The principle of volume selection is the same as with the double spin-echo sequence (Fig. 2). The difference is the process of signal production. The first 90° pulse is used to produce transversal magnetization within a selected slice identical to the 90° pulse within the PRESS sequence. While most of the spins are in phase immediately after the excitation, they begin to dephase with time under the influence of local inhomogeneities of the static magnetic field and applied field gradients. After a time TE/2, the second 90° pulse rotates the dephased magnetization within the *xy*-plane into the *zy*-plane. The axes *x* and *y*

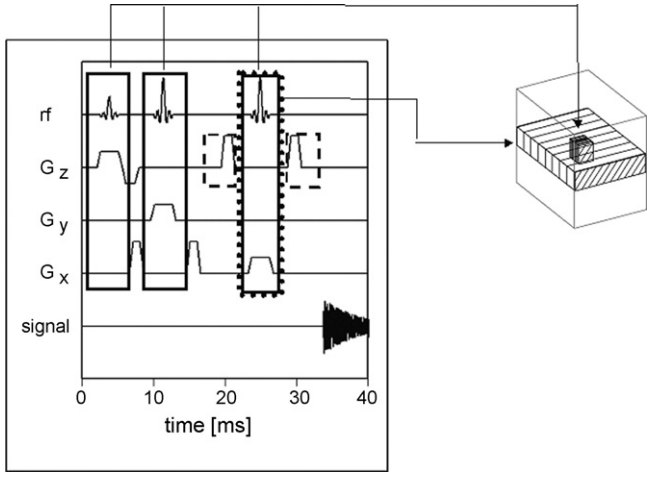


Fig. 3. Spoiling of unwanted signals in a PRESS sequence. The combination of the three rf pulses (marked by bold lines) leads to the excitation of the selected voxel, the unwanted signal contributions originating from last pulse(dotted), which excites the shown slice, can be strongly reduced by the additional spoiler gradients (dashed). The signal from the region of interest is not affected by the spoiler gradients due to the effect of the refocusing 180° pulse.

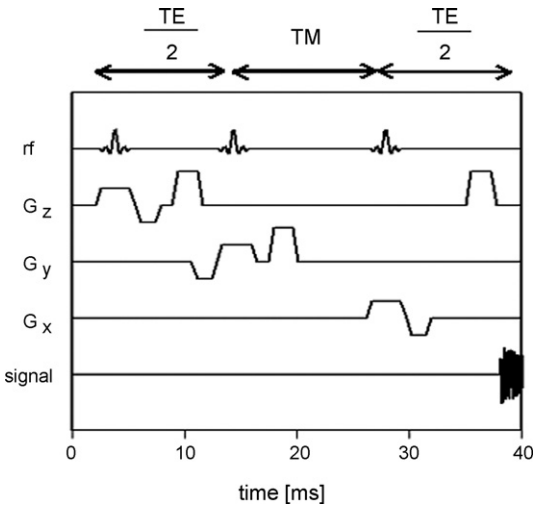


Fig. 4. Sequence scheme for the STEAM sequence. The refocusing gradients have to positioned before the second rf pulse and after the third rf pulse. Only the first part of the data acquisition time is shown.

are here used within a co-ordinate system, which rotates with the resonance frequency around the $z$-axis. Those components of the magnetization vectors, which remains in the transverse plane after the effect of the second rf pulse will experience a dephasing due to inhomogeneities and spoiler gradients and after some time, these components will cancel out each other. For the longitudinal magnetization, however, no dephasing occurs and therefore the $z$-components of the magnetization vectors are almost unchanged at the time, when the third 90° pulse is applied. The time between the second and the third pulse is called TM interval. During this interval, the z-components of the magnetization experience only T1-relxation, which is not strong since the usual time interval for TM is only few milliseconds. After the last 90° pulse, the remaining part of the magnetization is again flipped into the transverse plane. The sum magnetization after this pulse is zero, since the direction of the magnetization vectors varies between the $+x$ and the $-x$ direction and therefore the particular magnetization contributions are cancelled out. After the third pulse, a rephasing occurs similar as in conventional spin-echo sequences and at the time TE + TM all spins are again in the same phase and build a measurable magnetization vector. Due to the missing influence of that magnetization, which has been dephased within the TM-interval between the second and third pulse, the amplitude of the sum vector at the begin of the data acquisition is only half of that amplitude, which can be obtained in a spin-echo sequence with the same echo time [14]. An important part of the STEAM-sequence are the spoiler gradients, which are necessary to avoid signal contributions from regions outside the volume of interest. The last pulse in the STEAM-sequence is a 90° pulse, which lead to an excitation of all spins in the selected slice. A strong spoiler gradient after the last rf pulse dephases the transverse magnetization. For the spins within the volume of interest, the effect of the spoiler gradient is compensated by the action of a prephasing gradient between the first and second rf pulse. An additional spoiler is usually applied between the second and third rf pulse. In this time period all available magnetization does not contribute to the STEAM signal and should be dephased.

The main advantage of the STEAM sequence compared to PRESS is a reduced minimal echo time. The time between the second and the third rf pulse is part of the echo time in spin-echo sequences. In STEAM sequences, however, the relevant part of the magnetization vectors have only longitudinal magnetization in this time and experience only T1 relaxation, but no T2 relaxation. Since in human tissue T1 is mostly much longer than T2 in human tissue, the signal loss in the time between the second and third pulse is much less in STEAM sequences. This sequence is therefore used, if very short echo times should be realized. Another advantage of the STEAM sequence is the absence of 180° pulses. This limits the necessary peak rf power, which might be a problem at higher field strengths, and reduces of the difficulties with non-ideal slice profiles [15].

### 2.2. Artifacts in single voxel spectroscopy

One of the most important quality parameters in single voxel spectroscopy is the volume selectivity of the acquired signal. The measurement sequence must avoid any signal from the regions outside the volume of interest. The first tool to reach this aim is careful spoiling of unwanted signals. A strong spoiling is especially necessary, if small structures with relatively high signal intensity occur in the examination volume. In $^{1}$H-MRS examinations of the brain such critical structure is the subcutaneous fat along the skull. The fat layer is partly very thin, but gives a very strong signal compared to the metabolite signals to be examined. The sufficiency of the spoiling within a sequence for brain H-MRS can therefore be checked by the strength of unwanted contamination of the acquired signal by fat signals [16]. Since several crusher gradients are included in the sequences and various unwanted coherences are dephased differently, the order of the slice selection gradients has a pronounced effect on the overall performance of the sequence and hence the achieved spectral quality [17]. Another source of unwanted signal contributions arises from the fact, that rf pulses with a limited duration cannot excite a rectangular slice profile as desired. The real slice profile consists of a diminished excitation near the borders of the slice within the excited the slice and a small residual excitation near the borders of the slice outside of the excited the slice. Additionally, a minor oscillatoric excitation will occur even in a larger distance of the desired slice position. The oscillatoric behavior of this excitation, whose amplitude is often less than one percent, prevents usually contributions to the measured signal. The oscillatoric excitation results in signal contributions with positive and negative phases, which will cancel out each other in most cases. This excitation can nevertheless lead to artificial signals in the acquired spectrum, if the magnetization outside of the selected slice is much larger than that of the observed metabolites inside the slice. In this case even a very small excitation angle can lead to visible erroneous contributions in the spectrum. The fat near the skull might be one possible source for such a large magnetization. This artifact can be reduced by the application of regional selective outer volume suppression pulses.

A further problem in volume-selective spectroscopy is the chemical shift displacement. The origin of this artifact is the same as the chemical shift artifact in MR imaging: Since the localization of the selected slice is based on the frequency of the applied rf pulse and since the resonance frequency of protons in different molecular surrounding is different, the exact position of the selected slice depends on the resonance frequency of the protons. The spatial difference between excitation profiles for protons with a difference $\Delta\omega$ in the resonance frequency is $\Delta x = \Delta\omega/\gamma G$, where $\gamma$ is the gyromagnetic ratio and $G$ is the strength of the field gradient applied simultaneously with the rf pulse (Fig. 5a and b). This applies to all three excitations of a PRESS or STEAM sequence and leads to diagonal shift of the voxel position in dependence on the resonance frequency dif-

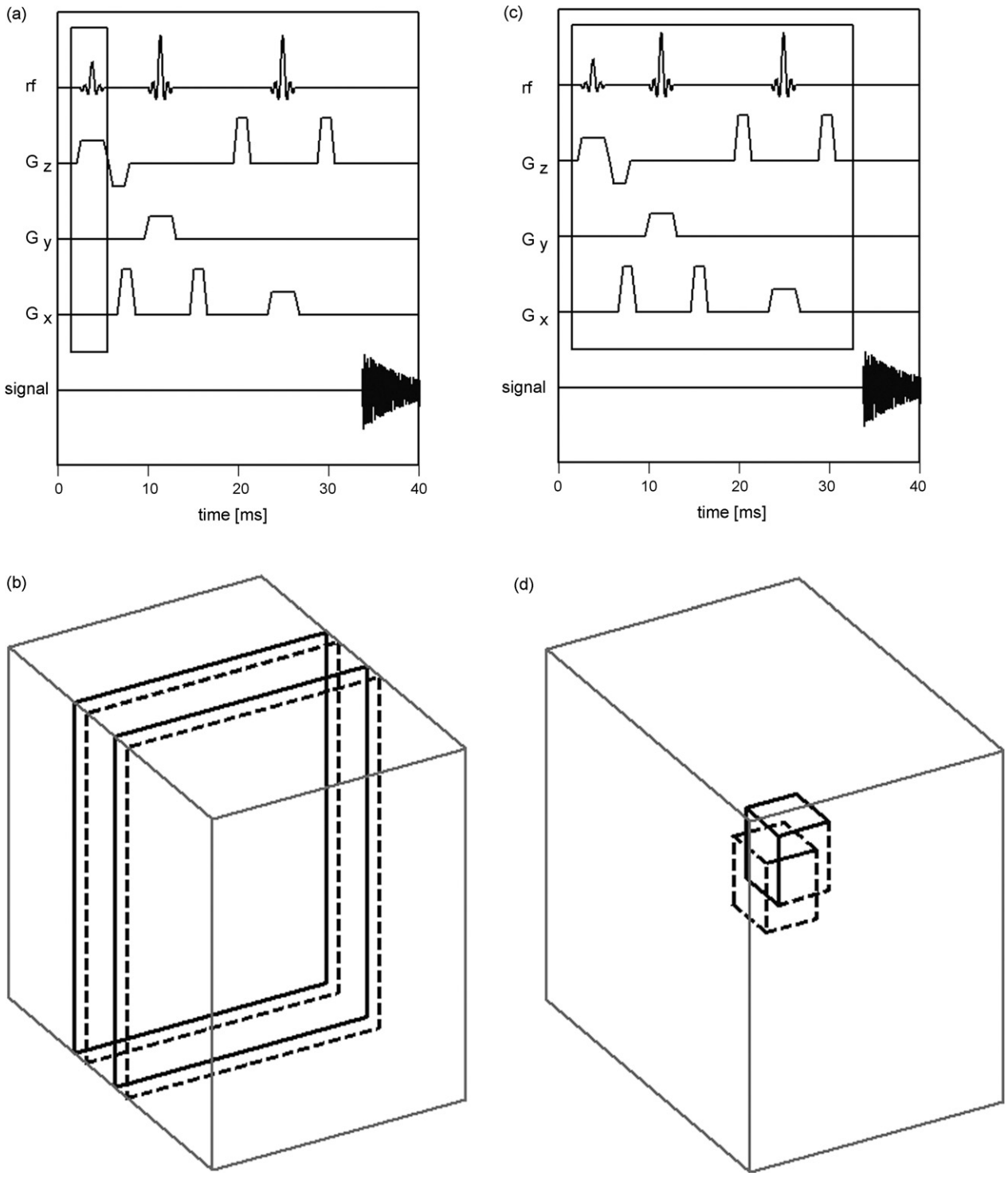


Fig. 5. The effect of two different resonance frequencies (bold and dashed) on the position of the excited volume for one (a and b) and three rf pulses (c and d) of a PRESS sequence.

ference of the protons (Fig. 5c and d). Since in $^{1}$H-MRS not the water signal but the signal of the metabolites is of interest, the resonance frequency of the MR system is often adjusted to the resonance frequency of one of the major metabolites (NAA in brain measurements). Nevertheless, it should be kept in mind that the exact localization of the selected region of interest is different for different metabolites as well as for water and fat signals. Especially for the selected voxel in the proximity of the skull, strong fat signal might occur, if the voxel position for the resonance frequency of fat is shifted towards the skull. The extent of the chemical shift displacement can be reduced by the use of strong field gradients for the slice selection.

The magnetization of all metabolites of interest in proton volume-selective spectroscopy is much smaller than the magnetization of water. The resonance frequency of water is a 4.7-ppm and has some spectral distance from the metabolite signals, which are usually in the range from 1 to 4 ppm. However, the wing of the water signal is very broad and therefore the water signal superimposes the signals from metabolites. This can be avoided by application of a frequency selective water suppression pulse (Fig. 6). The amplitude of this rf pulse is usually adjusted for a most efficient reduction of the water peak amplitude. Due to small variations of the amplitudes of static magnetic field and radio frequency field in the selected volume, the water suppression is often not perfect, but sufficient to reduce the broad peak foot of the water signal below the noise level. The amplitude of the unsuppressed water signal can be used for the quantification of metabolite spectra [18].

An important prerequisite for a meaningful spectrum is a sufficiently small line width of the spectral peaks. This line width depends on the transverse relaxation time and on the homogeneity of the magnetic field within the selected volume. The local variation of the magnetic field is different for each examined subject. The distribution of the magnetic field can be modified by variation of the electric current within the shim coils, which produce additional magnetic fields of different spatial shapes. This process is called the shimming and is usually performed as an automatic procedure during the preparation of a spectroscopic measurement. In some locations of the selected voxel, e.g. in the cerebellum, the automatic shimming procedure may lead to suboptimal results and a manual shimming can improve the results.

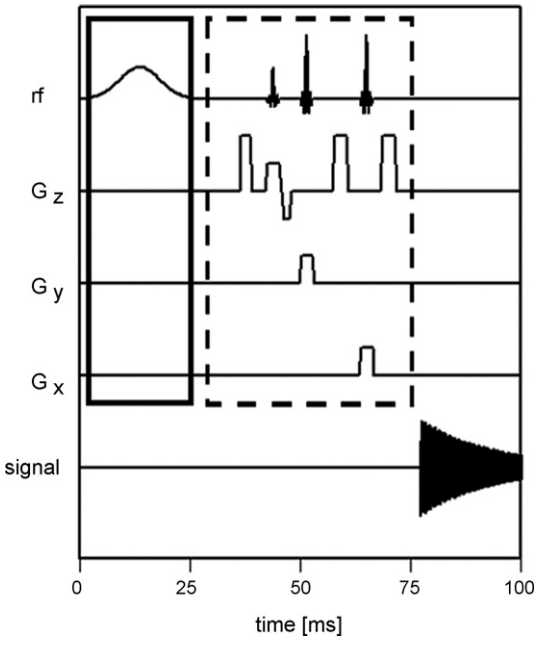


Fig. 6. The implementation of a water suppression pulse within a PRESS sequence. The additional pulse (marked by the bold line) is applied with a large pulse duration and without a gradient prior to the volume selection part (marked by the dashed line) of the sequence.

### 2.3. Coupling effects in single voxel spectroscopy

Most of the phenomena in MRI including the principles of volume selection in MRS can be explained by the simple model of magnetization vectors, which are aligned to the direction of the static magnetic field in the fully relaxed state. These vectors can be affected by the application of rf pulses and show a precession within the transverse plane with a frequency, which depends on the local magnetic field. The length of the vectors changes due to the influence of relaxation processes. The amplitude and the shape of the signal peaks of some metabolites in proton spectroscopy, like NAA, creatine and choline, can be completely explained with this simple model. Other metabolites, however, show a complex signal pattern, which not only depends on the chemical shift of the examined metabolites, but also on the interaction of the different protons within these metabolites. If more than one proton in a larger molecule contributes to the signal and if these protons are not magnetically equivalent, as e. g. the two protons in water, then a coupling between these protons occur. The main effect of coupling is a splitting of the resonance peak. Instead of only one single signal, signals at two or more resonance frequencies are obtained. The distance between these frequencies is described by the coupling constant *J* and contrary to the chemical shift, the coupling constant does not depend on the strength of the static magnetic field but is characteristic for a given molecule. The amplitude of the signals for each of the resonance frequencies, however, is strongly dependent on the measurement sequence. The shape of the complex signal pattern, which are obtained for molecules as lactate, glutamate, glutamine, GABA or glutathione can be calculated using a product operator formalism based on quantum mechanic principles [19], if the measurement parameters are exactly known. Most important for the shape of the signal pattern of a given metabolite is the timing of the used sequence (TE1 and TE2 in the PRESS-sequence, TE and TM in the STEAM-sequence) and the used flip angles. For a given combination of measurement parameters, the expected signal pattern can be calculated [20]. In in vivo measurements, the sequence timing is known, but the exact values of the applied flip angles are often uncertain, as the actual flip angles of the rf pulses at a selected position are mostly only near the nominal values, but not the exact ones. Furthermore it is not possible in volume-selective spectroscopy to apply pulses with an ideal slice profile. While in the center of a selected voxel the nominal flip angle might be nearly met, all possible flip angle values between the flip angle in the center of the voxel and the zero

value occur between the center and the borders of the voxel. The signal pattern of coupled system might therefore be different at different positions of the voxel and only the mixture of all these signals can be obtained in single voxel spectroscopy. The strong dependence of the signal pattern on the echo time of the used sequence was described in detail by Ernst and Hennig [21]. In in vivo measurements, additional superpositions with signals from other metabolites occur, which leads to an even stronger cancellation of signals. The observation of signals from molecules with coupled protons therefore often requires the use of short echo times in single voxel spectroscopy, however, the superposition of signals will remain a reason for difficulties in the separation of signals from different metabolites. This problem can be partly overcome, if editing sequences are used or series of sequences with varying measurement parameters are performed.

So-called homonuclear spectral editing techniques are characterized by additional frequency selective rf pulses within the sequence. These pulses can be used as a filter for signals from coupled spins, if additional signal subtraction techniques are applied [22]. This technique can be combined with PRESS or with STEAM sequences. Another possibility of an improved separation of different metabolites is the 2D-spectroscopy. In this case, the measurement is repeated several times with continuous change of a selected parameter, e.g. the echo time in a PRESS sequence or a STEAM sequence. While uncoupled spin will show the expected exponential signal decay with T2 as the time constant, phase and amplitude of signals of coupled spins are oscillating with a monotonously increasing echo time [23]. By an additional Fourier transformation the oscillating frequency, which is related to the coupling constant, can be mapped to a two-dimensional spectrum and a separation of the signal of coupled spins to several peaks can be observed [24]. This correlation spectroscopy (COSY) technique can also be combined with conventional techniques for volume selection. A major disadvantage of this technique is the long measurement time, which is necessary to acquire data not only at one, but at many different echo times.

### *2.4. Separation of metabolites and macromolecules*

In clinical MRS examinations, spectroscopic measurements with long echo times (e.g. 140 or 270 ms) are commonly performed. These spectra contain only of a few metabolites and a flat baseline and are therefore easy to analyze. In contrast, using short echo times (20–30 ms), additional signals can be obtained from metabolites with short T2 relaxation times or with *J*-coupling effects, but due the irregular baseline of the spectra which occurs at short echo times these data are more difficult to evaluate quantitatively. The sources of the irregular baseline are signal contributions from macromolecules, which have very short T2 relaxation times and therefore large line widths. These macromolecules are further characterized by shorter T1 relaxation times than metabolites. The signals from macromolecules can therefore be separated by introducing a T1-selective encoding of the signal. This can be reached by a non-selective inversion pulse and a appropriate time delay prior to the measurement sequence [25,26]. This inversion time TI can be adjusted to suppress either signals from macromolecules or from metabolites. The resulting macromolecule-nulled or metabolite-nulled spectra can be used for a separate quantification of both components [27].

## 3. Advantages and disadvantages for clinical applications

The most often used proton spectroscopy technique is the long echo time PRESS sequence. At echo times of 140 or 270 ms the obtained spectrum in the brain is easy to analyze: only signals from NAA, creatine, choline and – in pathological tissue – contribute to the signal, and all of them are clearly separated. Due to coupling effects, the signal from lactate is a doublet and it occurs with a negative amplitude at TE = 140 ms, which facilitate the confirmation of this metabolite. At shorter echo times, additional information can be obtained: the spectrum consists of additional signals from metabolites like glutamate, and glutamine. Due to coupling effects, these signals occur as multiplets and superimpose to other signals. The analysis is therefore more difficult. Further difficulties arise from an irregular baseline of the spectrum. Macromolecules with short T2 relaxation times and therefore very broad signal peaks modify the baseline beneath the metabolite signals. Short echo time proton spectroscopy needs therefore sophisticated evaluation strategies to get reliable values of the amplitude of signals from different metabolites [28]. If especially structures with very short relaxation times should be examined, the effect of the T2-decay can be reduced by using echo times as short as possible. Using STEAM sequences, echo times of 15–20 ms can be obtained, while the shortest available echo time in PRESS sequences is often 30 ms.

## 4. Conclusions

In the first years of whole body MR application, several techniques for volume-selective acquisition of magnetic resonance spectra from selected volumes within the body were proposed. Two of them are now widely used, PRESS and STEAM. They provide a reasonable selectivity of the desired volume. Possible spurious signals can be avoided by careful spoiling and outer volume suppression.

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