Optimization of $^{31}$P magnetic resonance spectroscopy in vivo

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Abstract. The main problem of magnetic resonance spectroscopy on phosphorus nuclei ($^{31}$P MRS) is low signal-to-noise ratio (SNR) of spectra acquired on clinical (3T) scanners. This makes quantitative processing of spectra difficult. The optimization of method on a single-voxel model reported in current work implicates an impact of the spin-lattice ($T_1$) relaxation on SNR, and also evaluates the effectiveness of Image Selected InVivo Spectroscopy (ISIS) pulse sequence modification for the increase of SNR.

1. Introduction

Magnetic resonance spectroscopy on $^{31}$P nuclei ($^{31}$P MRS) is a unique way of non-invasive investigations of energy and lipid metabolism of human and animal tissues in vivo, as well as a method of intracellular pH measurement. $^{31}$P MRS has demonstrated its high effectiveness in metabolic processes investigations in the norm and in pathology, including the studies of energy metabolism response to neurostimulation in local brain zones [1].

The $^{31}$P MR spectra of brain display signals for the phosphate groups of energy metabolism participants: phosphocreatine (PCr), ATP (3 peaks: b-, a- and g-ATP), inorganic phosphate (Pi); dinucleotides (DN) and the signals of lipid metabolism participants: phosphomonoesters (PME) phosphocholine (PC) and phosphoethanolamine (PC), phosphodiesters (PDE) glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE) and unidentified (GPX). From the Pi frequency measured relative to PCr, one can calculate the intracellular pH value [2].

When comparing with protons, phosphorus nuclei have ~2.5 lower gyromagnetic ratio. This creates the main $^{31}$P MRS in vivo problem: the necessity of increasing the volume of interest and spectra acquisition time to achieve satisfactory SNR values for the metabolites of interest. Such parameters of spectra obtaining like time of repeat (TR) and number of signals averaging (NSA) provide SNR gain by means of acquisition time increase. The TR parameter optimization for each particular study, as well as proton-phosphorus decoupling and nuclear Overhauser enhancement (NOE) allow to decrease the required time.

The aim of current study is to obtain $^{31}$P MR spectra with high SNR at minimal time of acquisition.
2. Materials and Methods
Eleven healthy subjects (6 male, 5 female) aged 18-50 became participants of this study. All subjects passed a standard diagnostic MRI examination at Philips Achieva 3.0T, including T₂, T₁, diffusion-weighted and FLAIR images. No structural abnormalities according to MRI data served as the study entry criterion.

The spectroscopy study was performed by using a transmit-receive head dual-tuned 31P/1H bird cage coil by Rapid Biomed, Germany. Keeping in mind the chemical shift displacement artifact [3], the spectroscopy volume of interest (voxel) sized 80x60x60 mm was located in such a way as to contain the most part of brain tissue. Voxel location is shown in figure 1. Voxel positioning was performed with the help of 3D localizer (survey) images and a set of 2D axial T₂-weighted images.

Pulse sequence (PS) ISIS was used for voxel localization, the parameters were as follows: echo time (TE)=0.1 ms, total magnetization vector flip angle (FA)=35°, number of spectral points=1024, spectral bandwidth (BW)=4000 Hz, number of dummy scans for steady-state magnetization settlement=2. For SNR increasing PS ISIS was modified with broadband proton irradiation for decoupling implementing during 31P signal registration via PS waltz 4 [4], and broadband proton irradiation before 31P excitation pulses for NOE implementing via PS waltz 16 [4].

The 31P MR spectra SNR optimization study consisted of two parts. In the first part a standard PS ISIS without proton irradiation at any time was used for 3 spectra obtaining with the same NSA=64 and TR values 2000, 3000 and 4000 ms. In the second part TR=3000 ms and NSA=64 were fixed, decoupling and NOE were used. Eleven spectra were obtained with varying NOE parameter mixing (mix) time, equal to 0, 250, 500, ..., 2500 ms. The total length of the study was ~45 minutes, which is acceptable for a person in MRI scanner.

Spectral processing was performed in Spectroview program. In both parts of the study, SNR of each resonance in spectrum was measured. In the first part, the SNR increase (in %) for each spectral line of each subject was calculated when TR was increased from 2000 ms to 3000 ms, from 3000 ms to 4000 ms and from 2000 ms to 4000 ms. In the second part, the SNR of each peak for different mix times were normalized on the corresponding values at mix time=0

Statistical analysis was performed in STATISTICA 12 using Mann-Whitney U-criterion to evaluate statistical significance of differences between groups.

3. Results
The SNR changes (in %) for all 31P spectrum peaks for each TR pair of the first part of the study are demonstrated in table 1. It is obvious that an increase of TR leads to the gain in SNR, but not for all metabolites and not for all pairs of TR this gain exceeds the one that could be achieved by increasing of NSA and using smaller TR.

The example of SNR dependencies (for b-ATP and Pi peaks) as a function of mix time is demonstrated on graphs, figure 2. The SNR increase with the growth of mix time is common for all metabolites. The SNR gain for ATP, PC, GPC, GPX, DN resonance lines slows down at mix time values
~1250-1750 ms. For PCr, Pi, GPE, PE graphs the SNR increase is observed up to 2250-2500 ms values of mix time. The maximal SNR gain by means of NOE is around ~30%.

Table 1. Average percentage increase in SNR (SD). * marks the statistically significant (p<0.05 according to Mann-Whitney criterion) increase, that exceeds the effectiveness threshold (see discussion).

| Peak | TR=3000 ms vs TR=2000 ms | TR=4000 ms vs TR=3000 ms | TR=4000 ms vs TR = 2000 ms |
|------|--------------------------|--------------------------|----------------------------|
|      | Threshold = 18.1%        | Threshold = 13.4%        | Threshold = 41.4%          |
| PCr  | 33.0 (4.7)*              | 11.2 (3.7)               | 44.8 (9.4)*                |
| γATP | 18.5 (5.7)*              | 7.1 (5.8)                | 27.5 (13.1)                |
| αATP | 16.4 (7.4)               | 6.7 (2.1)                | 29.6 (15.7)                |
| βATP | 15.9 (6.0)               | 7.2 (4.1)                | 32.1 (17.1)                |
| Pi   | 35.3 (11.2)*             | 18.5 (6.6)*              | 44.1 (15.5)*               |
| PE   | 30.1 (13.6)*             | 11.8 (10.2)              | 56.6 (19.2)*               |
| PC   | 23.4 (7.1)*              | 4.1 (3.1)                | 42.1 (27.4)*               |
| GPE  | 37.7 (17.9)*             | 20.2 (9.5)*              | 73.6 (28.4)*               |
| GPC  | 29.3 (16.1)*             | 11.0 (8.8)               | 66.5 (31.0)*               |
| GPX  | 15.0 (11.5)              | 11.3 (5.1)               | 37.9 (19.8)                |
| DN   | 20.1 (5.8)*              | 9.4 (4.5)                | 22.3 (10.5)                |

4. Discussion
The larger TR value, the smaller NSA must be to keep spectrum acquisition time constant. SNR is proportional to the square root of NSA, so, when coming to TR=3000 ms from TR=2000 ms NSA has to be decreased by 33.3%, that automatically decreases SNR by 18.1%. This value is the effectiveness threshold: if, when NSA is kept constant, SNR gain by means of TR increase from 2000 ms to 3000 ms exceeds 18.1%, the usage of larger TR is profitable. The effectiveness thresholds are calculated analogously in other cases: it is 13.4% when changing TR=3000 ms to 4000 ms, and 41.4% when TR=2000 ms changes to 4000 ms.

Table 1 demonstrates that TR = 3000 ms is more preferable than TR = 2000 ms: the SNR gain exceeds the threshold for most of metabolites. It is connected with the fact that the time required for the full relaxation of 31P spectrum metabolites (T1) is significantly higher than TR=2000 ms [5]. A comparison of TR=4000 ms with TR=3000 ms shows that SNR gain for most of metabolites is not enough to compensate the loss in signal owing to NSA decrease. This statement is false for Pi and GPE peaks, and that agrees with the fact that T1 values of Pi and PDE are the ones of most large.

TR=4000 ms comparison with TR=2000 ms demonstrates the effectiveness of the larger TR for groups of PME and PDE peaks, PCr and Pi, but not to ATP and DN peaks. For that reason TR=4000 ms...
should be used in case of necessity to provide the maximal SNR\textsubscript{Pi} and when lipid metabolism is of interest in the study. SNR\textsubscript{Pi} is crucial for intracellular pH determination, because the variation range of this parameter in vivo is rather narrow [6]. Local brain pH changes owing to no damaging exposure, for example, neuronal activation, are ~1% [7], hence, may be missed because of low SNR\textsubscript{Pi}.

In summary, TR=3000 ms is universally applicable. That contradicts [8], where authors state that SNR of 3T brain \textsuperscript{31}P spectra is insufficient when TR<4000 ms, but, unfortunately, no quantitative analysis of results is shown. We show that change of TR from 3000 ms to 4000 ms does not give profitable SNR gain for most of metabolites, although it is recommended in some cases. What is more, for the alone energy metabolism analysis TR≤2000 ms with not much increase of NSA may be used, that allows to shorten the study time. For example, in the study of energy metabolism response on visual cortex stimulation in schizophrenia patients [1], where the fastest data acquisition was required, we revealed energy metabolism alterations using TR=1200 ms.

The larger TR, the longer it is possible to use proton irradiation before \textsuperscript{31}P excitation pulse. By means of NOE during this excitation the difference increases between populations of \textsuperscript{31}P nucleus energy levels. The maximal value of heteronuclear NOE theoretically achieved is half of gyromagnetic ratios ratio, which means ~150% [9]. But in reality it is impossible to achieve such values: NOE enhancement monotonically rises with the mix time increase until the plateau, meaning equilibrium condition [10].

The time of the plateau beginning is strongly dependent on system parameters, particularly on participating nuclei and constant magnetic field strength [10]. According to the graphs on figure 2, noticeable SNR gain for most of metabolites ends at ~1500 ms of mix time. PCr, Pi, PE, GPE are exceptions, the increase is going on up to ~2500 ms. Maximal mix time is defined by TR parameter. At the universally applicable TR=3000 ms ~500 ms are required for \textsuperscript{31}P signal registration, so mix time is constrained by ~2500 ms. This value is enough for a maximal SNR gain by means of NOE practically for all metabolites. When using TR=2000 ms for energy metabolism investigations, available maximal mix time ~1500 ms is enough for maximizing NOE on ATP resonance lines.

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