Dynamic 31P-MRS during visual stimulation protocols in healthy young adult subjects

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Introduction

Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS) allows the non-invasive study of human brain metabolism. Measurable metabolites in ^{31}P spectra may be either related to energy metabolism (PCr, α-ATP, β-ATP, and γ-ATP, Pi and NAD+NADH) or have structural functions (PE, PCh, GPE, GPCh). Also, pH and Mg^{2+} concentration are measurable parameters which may provide additional metabolic information. ^{31}P-MRS combined with visual stimulation has been widely used to investigate energy metabolism in healthy subjects and patients suffering from diseases such as Parkinson's or Bipolar Disorder. However, reported literature findings have been controversial, thus it is necessary to evaluate the combined variability from subjects and from the technique in resting condition. In this work we used a large number of subjects to assess metabolic (intra and intersubjects) variability in resting conditions and metabolic changes resulting from two visual stimulation protocols. We used parametric tests to evaluate the statistical significance of results.

Aim: To investigate metabolic variability during rest and metabolic changes resulting from different visual stimulations on healthy subjects.

Materials and Methods

^{31}P spectra were acquired in a 3 T MR System (Achieva, Philips, The Netherlands) with a 14 cm diameter surface coil. An excitation acquisition non-localized sequence was used, with TE/TR=0.9/3750 ms, 8 NSA and 8 phase cycles, bandwidth of 3 kHz and 1024 points. Automatic shimming, NOE enhancement and decoupling were also performed. Rest study: 10 healthy subjects (8 males, age 20-25 years old), awake and eyes closed during acquisition (total duration 7.5 min). Dynamic study: two different protocols were tested. Short protocol: 20 healthy subjects (9 males, 20-25 years old), visual stimulation consisted of three blocks *off* (no stimulation) and two blocks *on* of 1.5 min each (3 spectra/block, total duration 7.5 min). Long protocol: 18 healthy subjects (9 males, 18-37 years old), same stimulation paradigm as short protocol but with 5 min block duration (10 spectra/block, total duration 25 min). Visual stimuli consisted of a black-white radial checkerboard pattern flickering at 8Hz and were delivered by an Invivo's Eloquence™ system. All subjects gave written consent. Spectra were processed using the AMARES algorithm from jMRUI (v3). Mean relative changes (regarding the 1st *off* block) for *on* and *off* states were computed for every metabolite for every subject. Paired t-tests were performed to evaluate significant alterations between *on* and *off* blocks in the dynamic study.

Results and Discussion

Spectra from each block were added creating one representative spectrum for each block. The signal-to-noise ratio achieved allowed identification of NAD+NADH and Pi (intracellular at 4.81 ppm and extracellular at 5.27 ppm) resonances (Fig. 1) for short and long protocols. In 80% of subjects a resonance at -0.5 ppm was observed, with relative amplitude to PCr of 0.22±0.16. This resonance is not well reported in human brain literature [1] and it is attributed to guanidineacetate (PGAc).

Rest study: Intrasubject (or technique) variability was evaluated by dividing acquisitions in five 1.5 min blocks and calculating the mean coefficient of variation (CV = standard deviation/mean) of every metabolite over subjects (δ) (Table 1). Intersubjects variability was evaluated by normalizing individual metabolite areas with respect to the total spectrum area and calculating the corresponding CV (μ). Metabolites with lower signal intensity showed larger intrasubject deviations, such as β-ATP and GPE. Deviations due to intersubjects' variability were superior to deviations due to the technique (intrasubject). The mean pH calculated was 7.04±0.01, which is consistent with results from the occipital region reported in the literature [2]. The Mg^{2+} concentration calculated was 0.13±0.01 mM, which is inferior to the 0.37±0.12 mM value reported from prefrontal cortex of healthy subjects [3].

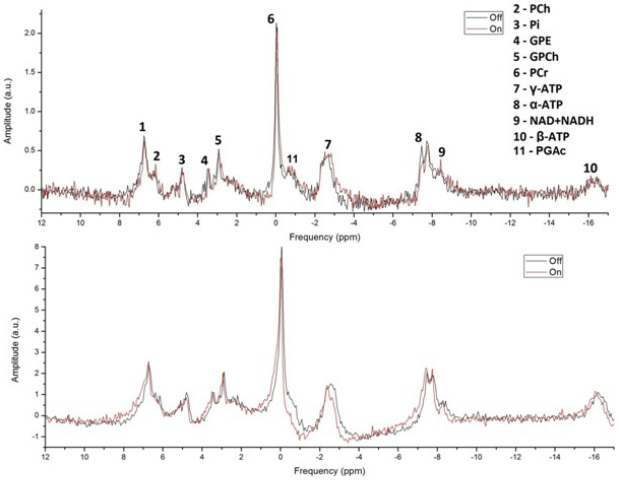

Fig. 1. Examples of resulting spectra from a block off and a block on from the (top) short and (bottom) long protocol.

Dynamic study: Both protocols showed significant differences among *on* and *off* states for Pi (higher in *on* blocks) and for the PCr/Pi ratio (lower in *on* blocks), with a mean relative change among these states of 13.4% (Pi, p=0.04) and -17.4% (PCr/Pi, p=0.007) for the short protocol and 5.3% (Pi, p=0.009) and -5.9% (PCr/Pi, p<0.001) for the long protocol. This is the main result from ^{31}P-MRS reported in the literature [4,5], although several studies used a reduced number of subjects for statistical testing. The higher decrease of the PCr/Pi ratio in the short protocol demonstrates the PCr function of providing energy during a short term period after stimulation. Also, for the long protocol both the α-ATP and PE resonances were found to be slightly, but significantly, different among blocks (higher in *on* blocks), with mean relative changes of 1.5% (α-ATP , p=0.01) and 1.2% (PE, p=0.048). No changes on pH and Mg^{2+} concentration were observed between rest and stimulation periods.

	GPCh (%)	β-ATP (%)	α-ATP (%)	γ-ATP (%)	PCr (%)	Pi (%)	PCh (%)	PE (%)	NAD+NADH (%)	GPE (%)	PCr/Pi (%)	PGAc *(%)
δ	6.4	18.7	3.4	4.9	3	9.3	9.3	3.8	8.8	26.6	19.7	74
μ	24	44.9	34.6	24.8	8.9	35.3	20.5	12.8	28.5	51.7	29.2	17.9

Table 1. Coefficients of variation from metabolites analyzed during rest study. δ represents variability from the method and μ represents variability between subjects. * Data from 8 subjects.

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Acknowledgments

This work was performed at HC USP-RP and HC UNICAMP, supported by FAPESP.