Metabolic alterations in rat somatosensory cortex during forepaw stimulation measured by ^{1}H-MR spectroscopy at 11.7 T

S. Xu[1], J. Yang[1], C. Q. Li[1], W. Zhu[1], J. Shen[1]
[1]NIMH, Bethesda, MD, United States

Introduction

The metabolic events associated with neuronal activity can be measured *in vivo* using fMRS. Many groups have reported the effects of brain activation on phosphocreatine (PCr) and lactate (Lac) in human and animal brains. However, the results have been controversial, owing to the low intrinsic SNR and/or spectral resolution associated with ^{31}P MRS and ^{1}H MRS at low field strength. In this study, we employed ^{1}H MRS at 11.7 T to investigate whether cerebral metabolism in rat somatosensory cortex responds to continuous forepaw stimulation. Single-shot 64 x 64 EPI-based BOLD fMRI experiments were also performed as a reference.

Methods

Male adult Sprague-Dawley rats (170-210 g, n=11) were anaesthetized using α-chloralose throughout the fMRI and ^{1}H MRS experiments. Both forepaws were stimulated through needles inserted in-between digits 1, 2 and 3, 4 under the skin, respectively. The needles were connected to an electrical stimulator that generated square pulses (2 mA current/0.3 ms duration/3 Hz frequency). All experiments were performed on a Bruker 11.7 T AVANCE spectrometer with an 89 mm i.d. vertical-bore magnet. A ^{1}H surface coil of 15 mm i.d. was used. The coil was positioned 0-1 mm rostral to bregma. Adjustment of shims was accomplished with FASTMAP/FLATNESS. A 3.5 x 2.0 x 4.5 mm^3 volume was chosen on the right side of the somatosensory cortex for localized spectroscopy. Spectra were obtained with 4096 data points/sw=4000 Hz/TR=5 s/na=128. For fMRS, the electrical stimulator was set in the continuous mode during the 11 minutes acquisition. For fMRI experiments, three-slice single-shot coronal spin-echo EPI was used (st=2 mm/FOV=2x2 cm^2/matrix= 64x64/TR=500 ms/TE=25 ms). For fMRI, stimulation paradigm consisted of 15 s baseline/20 s stimulation/15 s baseline. MRS data were analyzed using LCModel and Matlab. The concentration of PCr+creatine (Cr) (8.5 µmol/g ww) was used as an internal reference standard. Student's t-tests were used for the statistical analysis. Activation maps from fMRI experiments were generated using STIMULATE with the threshold of cross-correlation coefficient (ccc) set to 0.25 or 3.0.

Results

A typical pair of ^{1}H spectra before and during the forepaw stimulation is shown in Fig.1a and b. The corresponding fMRI image with the location and size of the spectroscopy VOI is shown in Fig.1c. PCr/Cr was significantly decreased by 13.4% ($p<0.01$) (Fig1d.). Among a total of 11 animals, 10 of them showed significant brain activation with ccc threshold of 0.3 and 1 with a ccc of 0.25. The responses of other metabolites to focal activation are also shown in Fig 2. Compared to pre-stimulation, there were significantly decreases in [PCr], [glutamate] (Glu), [*myo*-inositol] (MI) and increases in [Cr], [glutamine] (Gln) during stimulation. There are no significant changes in [aspartate] (Asp), [glucose] (Glc), [taurine] (Tau), [phosphorylethanolamine] (PE), and [*N*-acetylaspartate] (NAA) between the two states. Due to the inaccuracy of the LCModel method on metabolites of very low concentrations, results from alanine, aspartate, γ-aminobutyric acid, Lac, *scyllo*-inositol, glutathione, *N*-acetylaspartylglutamate, phosphorylcholine and glycerophosphorylcholine were excluded (No large accumulation of Lac was observed during stimulation (Fig.1)).

Discussion and Conclusion

Compared to previous ^{31}P MRS investigations, our ^{1}H MRS technique at 11.7 T offers a direct way to characterize the PCr/Cr system. We successfully resolved PCr and Cr peaks which have a chemical shift difference only of 0.01 ppm. Our results confirmed that [PCr] decreases during neuronal stimulation in the brain. The Lac signals in our study were lower than 0.3 µmol/g ww both before and during stimulation. The results indicated that there were no detectable or significantly large increases in [Lac] during stimulation. MI is at the center of inositol-polyphosphates (Ins) metabolic pathway. Ins act as critical second messagers in signal transduction. Although, the concentrations of Ins are below the detection limit for *in vivo* ^{1}H MRS, free MI which can be detected by ^{1}H MRS is a storage for Ins. Also, MI and various Ins resonate at different frequencies in ^{1}H MRS. A decrease in MI could be due to conversion of MI to Ins during the external stimulation. Glu is the major excitatory neurotransmitter found in the mammalian CNS. Studies have indicated that Glu released by neurons is taken up by glial cells and converted into Gln by Gln synthetase, which is exclusively localized in glia. The decrease in the [Glu] could be due to an imbalance in the Glu homeostasis process caused by increased glutamate release during focal activation. Since Glu is converted into Gln by Gln synthetase, the decrease in [Glu] should be correlated to an increase in [Gln]. Interestingly, we found no statistically significant difference between $|\Delta[Glu]|$ and $|\Delta[Gln]|$. The results indicate that PCr, Cr, Glu, Gln and MI react to focal functinal activation of the brain and may play important roles in short term brain activations evoked by external forepaw stimulation.

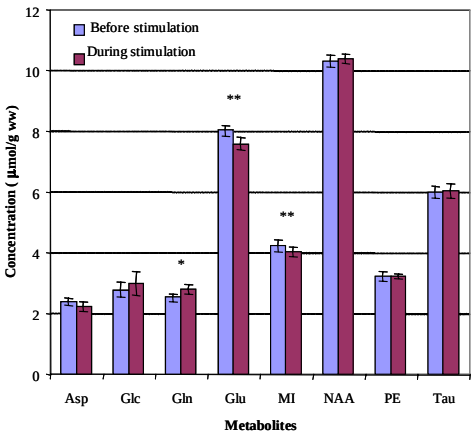

Fig2. Concentration of metabolites in the rat cortex before and during stimulation resulted from LCModel analysis. Error bars denote SEM. *p<0.05, **p<0.01.

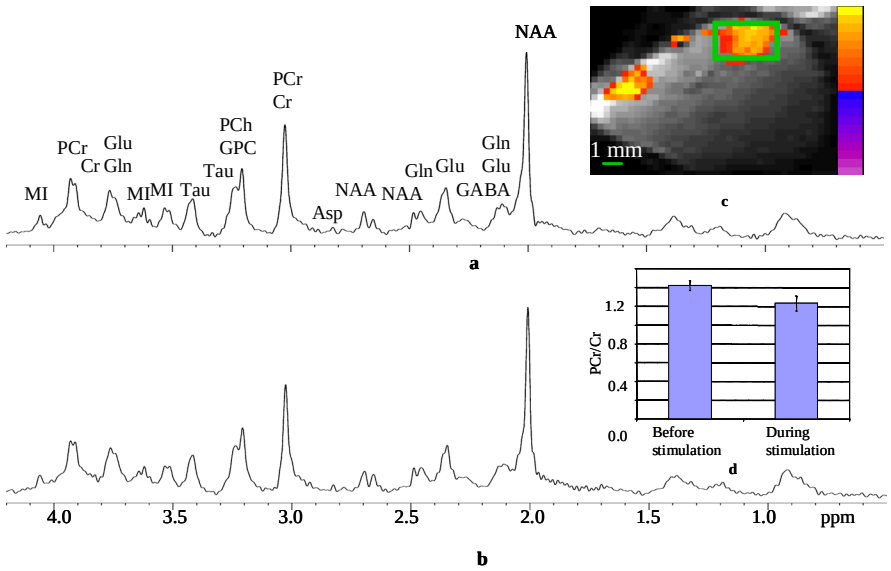

Fig1. *In vivo* ^{1}H spectra of the rat cortex from the VOI. **a**. Before stimulation. **b.** During stimulation. **c**. Activation map of the fMRI experiment. **d**. PCr/Cr.