Measurement of Metabolite concentration changes in the rat barrel cortex during sustained trigeminal stimulation

Nathalie Just[1], and Rolf Gruetter[1,2]
[1]*LIFMET, CIBM/EPFL, Lausanne, Switzerland,* [2]*Department of Radiology, Universities of Lausanne and Geneva, Switzerland*

Introduction: To date, only a couple of functional MR spectroscopy (fMRS) studies were conducted in rats [1, 2]. They demonstrated increased oxygen consumption [2] and significant changes in the phosphocreatine/creatine ratio but no changes in lactate levels [1]. This last result is controversial since either significant Lac increases [3] or no Lac changes have been observed during stimulation [4]. Due to the low temporal resolution of ^{1}H-MRS techniques, prolonged stimulation paradigms are necessary for investigating the metabolic outcome in the rat brain during functional challenge. However, sustained activation of cortical areas is usually difficult to obtain due to neural adaptation. Anesthesia, habituation, and contaminations due to unwanted contributions from lipids outside the selected voxel of interest, high variability of the basal state metabolites concentrations as well as low concentrations of the metabolites of interest (lactate (Lac), glucose (Glc) or γ-amminobutyric acid (GABA)) and small expected changes of metabolite concentrations need to be addressed.

The whisker barrel system offers an excellent model to study BOLD activation processes [5]. The rat barrel cortex can be reliably and reproducibly activated through trigeminal nerve (TGN) stimulation [6, 7]. Moreover, sustained barrel cortex BOLD activation was shown during prolonged whisker stimulation [8, 9].

For an accurate understanding of the link between metabolic and functional mechanisms in the brain, it is essential to develop BOLD fMRI and fMRS methods in conjunction with appropriate functional paradigms.

The aim of the present study was to evaluate the metabolite concentration changes in the rat barrel cortex during sustained trigeminal nerve stimulation.

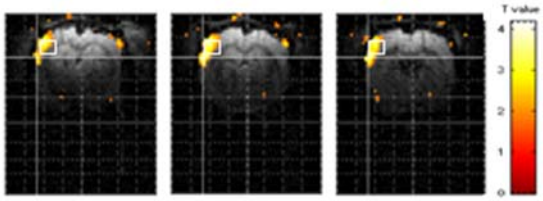

Figure1:T-maps for 3 consecutive slices showing BOLD activation in the barrel cortex of the rat during TGN stimulation. Location of the voxel of interest for 1H-MRS.

Materials and Methods: Animal preparation: Male adult Sprague-Dawley rats (n=6, 350±40g) were initially anaesthetized with isoflurane in a mixture of O_2. Each rat was orally intubated. A femoral artery and a femoral vein were catheterized for α-chloralose administration and blood gas sampling. After fixing the rat head using ear and bite bars, the rat was positioned in a dedicated holder. The breathing rate was monitored simultaneously with body temperature throughout the experiment with a rectal probe. Body temperature was maintained at 37.5ºC ± 0.5ºC with temperature-controlled circulating water placed under the rat. The blood pressure was monitored through a transducer attached to the catheterized femoral artery. Blood gases were sampled every 30 minutes and blood parameters were maintained at physiological levels (pH = 7.35-7.4, pCO2 = 39-45 mmHg and mean arterial blood pressure = 90-130mmHg) throughout the experiment. After surgery, anesthesia was switched from isoflurane to α□-chloralose; an initial intravenous dose of 80mg/kg was administered followed by a continuous intravenous infusion of 27mg/kg/h at a rate of 2ml/hour. **Trigeminal nerve stimulation:** Two stainless steel electrodes were percutaneously inserted either in the left or right trigeminal nerve. The cathode was inserted in the hiatus infraorbitalis as described in [7] and the anode was inserted either in the masticatory muscles. Electrical stimulation of one trigeminal nerve was performed by delivering square pulses using an external stimulator (1Hz, 2-3mA) [7]. **fMRI protocol:** All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian, Abingdon, UK) with 12 cm gradients (400mT/m in 120μs) and a quadrature T/R 17mm surface coil. First and second order shims were adjusted using FASTMAP [10] resulting in water linewidths of 12-15Hz in a 216μl volume. The BOLD response was assessed using single shot gradient echo EPI (TR/TE=2500-2000/25ms; FOV=20x20mm; matrix=64x64; slice thickness = 1mm; 5-6 slices, Bandwidth=325 KHz, 300-960 volumes). Each rat was exposed to three alternate periods of 10 minutes of rest and 10 minutes of TGN stimulation. **fMRS:** Localized Proton spectroscopy was performed using SPECIAL [11] in a VOI localized in the activated barrel cortex and after adjusting once more the shims using FASTMAP (12-15Hz). The unsuppressed water signal was measured at the end of the experiment. The voxel size for 1H-MRS was 2x2x4mm^3 and was positioned on the BOLD activation maps (motion-corrected cross-correlation maps with a cross-correlation coefficient >3 or motion-corrected T-value maps (GLM model) with t>3) obtained after a 10-minute TGN stimulation (fig.1). The raw 1HMRS spectra corrected for frequency drift and summed were used for LCmodel analysis [12] with a basis set of 21 simulated metabolites. For metabolite concentration time courses, blocks of 16 fids were summed using a moving average (3 x 16 fids ~ 1 minute, $SNR_{LCmodel}$> 8; Full width at half maximum<0.040ppm) over each 10-minute period per rat and then further summed over all the animals. Statistics were performed using a paired t-test. A pvalue <0.05 was considered significant.

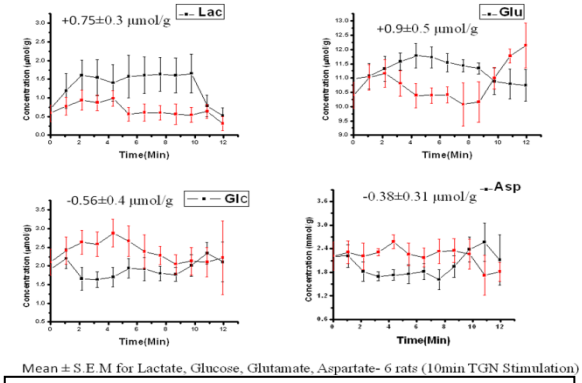

Figure2-a-d: Time-courses of Lac, Glu, Glc and Asp during 10min TGN stimulation and 10-min rest. Each point corresponds to 48 spectra (average over 3 scans or moving average) and averaged over 6 rats. The average difference between metabolite concentrations during stimulation and rest is indicated at the top of each curve.

Results and Discussion: The location of the voxel of interest was carefully chosen to avoid lipid contamination. When distortions were found at 1.3ppm in the lactate region, data were entirely discarded from the study. To evaluate whether TGN stimulation was effective or not, the total Creatine peak height was measured during rest and stimulation periods. An average increase of 1.7% of the peak height due to the BOLD effect [13] was observed in the activated barrel cortex. Metabolite concentrations were considered acceptable for CRLB <50%. The time courses for 6 rats of Lac (CRLB ~<30%), Glu (CRLB ~<5%), Glc (CRLB ~<30%) and Asp (CRLB ~<40%) are plotted (fig.2.a-b-c-d) for a- 10 minute TGN stimulation period and a 10-minute rest period. The average difference (± standard deviation) is given at the top of each curve. The average metabolite concentrations during TGN stimulation and rest for Lac, Glu, Glc and Asp were compared (fig.3). [Lac] (p=0.0001) and [Glu] (p<0.002) increased significantly during sustained TGN stimulation relative to the rest period while [Glc] (p<0.003) showed a significant decrease. Aspartate also demonstrated a tendency to decrease during TGN stimulation (p=0.08). Previous changes in metabolite concentrations measured by fMRS were reported in humans during visual stimulation [13]. The present study reports for the first time [Lac], [Glu], [Glc], and [Asp] changes measured during functional challenge in a rat models. These changes are in agreement with previous measurements performed during sensory stimulation in rats [14]. For the first time, the dynamics of metabolite concentration during sustained somatosensory activation in the rats using fMRS were evaluated.

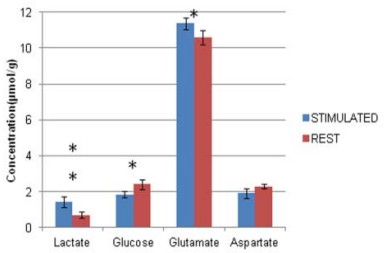

Figure 3: Comparison of [Lac], [Glc], [Glu] and [Asp] during stimulation and rest periods, ** p<0.0001; *p<0.003

References and Acknowledgements:[1] Xu S *et al*. Neuroimage. 2005;28(2):401-9.[2] Yang J *et al*. Neuroimage. 2006;32(3):1317-25. [3] Frahm J. *et al*. Magn. Reson. Med. 35, 143– 148. [4] Boucard C *et al*. Eur. Radiol. 15, 47–52.[5] Petersen C. Neuron 2007; 56:339-355. [6] Nielsen AN *et al*. Journal of Physiol 2001; 533: 773-785.[7] Just N *et al*. Magn Reson Imaging. 2010;28(8):1143-51.[8] Quairiaux C *et al*. J Neurophysiol. 2007 Mar;97(3):2130-47. [9] Just *et al*. ISMRM 2011-1590 [10] Gruetter R *et al*. Magn. Reson. Med. 2000;43:319-323.[11] Mlynarik V *et al*. Magn Reson Med 2006; 56:965-70. [12] Provencher S.W. (1993). Magn. Reson. Med.; 30(6):672-679.[13] Mangia S *et al*.J Cereb Blood Flow Metab. 2007b;27(5):1055-63. [14]Dienel G *et al*. J Cereb Blood Flow Metab. 2002; 22:1490-1502. Supported by the CIBM of the UNIL, EPFL, HUG and the Leenards and Jeantet Foundations.