Functional Magnetic Resonance Spectroscopy of the rat barrel cortex

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Introduction: Until now, unraveling metabolic changes during brain activation remains difficult. Mangia *et al* (1, 2) demonstrated that measurement of the neurochemical profile of the human primary visual cortex during sustained visual stimulation is possible. In rodents, prolonged activation of the somatosensory cortex is limited due to anesthesia and habituation. Since Hyder *et al.(3)* showed a one hour BOLD activation of the rat primary somatosensory cortex upon forepaw stimulation and concluded that oxidative glycolysis is the primary source of energy during cortical activation, investigations have been challenging and controversial (2, 4).Trigeminal nerve stimulation (TGN) allows investigating the BOLD fMRI activation of the rat barrel cortex (5). Here, functional proton MRS was conducted during sustained TGN stimulation allowing measurement of metabolic changes during barrel cortex activation.

Materials and Methods: Male SD rats (n=15 for fMRI; n=10 for fMRS, 350±40g). Orally intubated; Catheterized for α-chloralose and physiology control (pH ~7.4, pCO2~39-mmHg, MABP ~130mmHg, Temperature =37.5ºC ± 0.5ºC). All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian) with a quadrature T/R 17mm surface coil. First and second order shims were adjusted using FASTMAP (6). fMRI was performed as described in (5, 7). **fMRS:** Localized ^{1}H-MR spectroscopy was performed using SPECIAL(8) in a 2x2x4 mm^3 VOI localized in the activated barrel cortex and after re-adjusting the shims using FASTMAP. Blocks of fids were summed over each 10-minute period per rat and then summed over the 10 animals resulting in a stimulation spectrum (Fig2A) and a rest spectrum (Fig2.B). Spectra contaminated by extra-cerebral lipid signals were discarded. Metabolite concentrations were calculated using LCmodel (9). Statistics were performed using a paired-test. A pvalue <0.05 was considered significant.

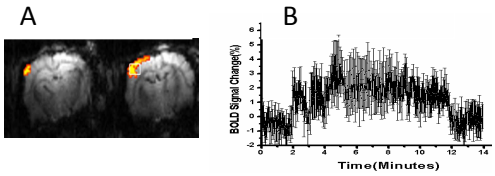

Figure1: A. Representative functional map (GE-EPI, TE=25ms, TR=2.5s, FOV=21x21mm, 64x64) during 10-minute TGN stimulation; Activated pixels correspond to cross-correlation coefficients >0.3. B. BOLD signal change averaged over 15 rats during a 10-minute TGN stimulation.

Results and Discussion: Reproducible 10-minute BOLD fMRI time-courses were obtained in rats upon sustained TGN stimulation. Fig1B shows the mean BOLD percent signal change over 10 minutes in 15 rats (Mean±SEM). An example of functional map overlaid on a single-shot GRE-EPI image is shown (Fig1.A) showing lateralized activation. In the VOI corresponding to the activated area in the barrel cortex (Fig1.A), shimming resulted in water linewidths of 15-19 Hz. The difference spectrum resulting from the subtraction of spectra in Fig2.A-2.B, is shown in Fig2C. An exponential multiplication corresponding to 0.6Hz line broadening was applied to the stimulation spectrum to match the linewidth of the rest spectrum before subtraction. The BOLD-free difference spectrum is shown in Fig2.D. Fig.3 shows an enlarge version of Fig2D between 1 and 3ppm. Positive Glu (2.15, 2.4 ppm) and Lac (1.32ppm) peaks are shown with red arrows. Negative Gln (2.46ppm) and Asp (2.6 and 2.8ppm) are shown with black arrows. Spectra were averaged across subjects and LCmodel quantification was performed. During stimulation, lactate (Lac:+42%; +0.32±0.01µmol/g) and Glutamate (Glu: +4.2%; 0.45±0.02µmol/g) increased significantly ($p<0.01$) whereas Glutamine (Gln: -8%;-0.3±0.01µmol/g) and Aspartate (Asp: -13%;-0.3±0.02µmol/g) showed a tendency to decrease although not significantly. However, Glucose (Glc) levels were unchanged. This result can be attributed to the variability between subjects. Using sustained TGN stimulation, concentration changes of Glu and Asp in the rat barrel cortex were in agreement with concentration changes observed by Mangia et al. (2) (Glu=+3%; Asp=-15%) during stimulation in the human visual cortex.

Conclusion: Sustained TGN stimulation is possible in the rat barrel cortex to study neurochemical consequences of activation.

Figure2

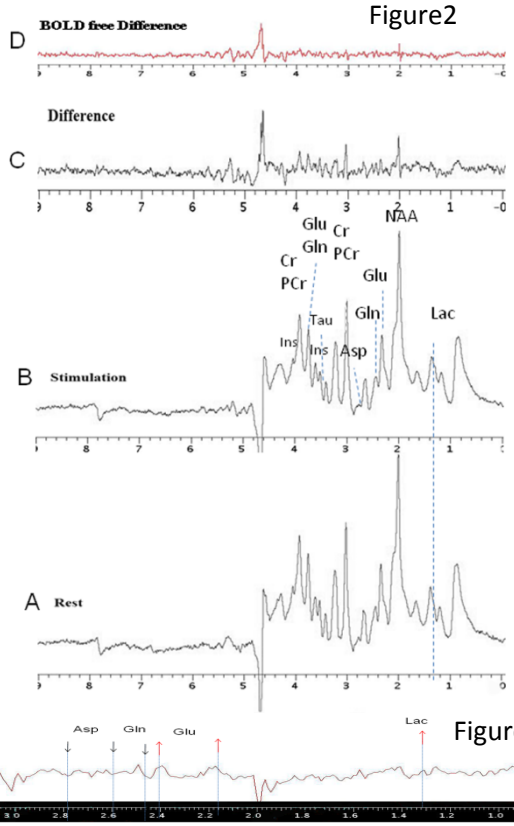

Figure3

Figure2 : Summed 1H NMR spectra during the rest period (A) and during the stimulation period (B) and corresponding difference spectrum (C) (10 rats). (D). BOLD free difference spectra after 0.6Hz line broadening. Arrows indicate Glc negative peaks. **Figure 3.** BOLD free Difference Spectrum between 1.0 and 3.0 ppm indicating Asp and Gln negative peaks (Black descending arrows) and Glu and Lac positive peaks (Red ascending arrows).

References:1.Mangia S et al. JNR.2007;85:3340-3346. 2.Mangia S et al. JCBFM. 2007;27:1055-1063. 3. Hyder F et al. PNAS; 1996; 93:7612-7617.4. Xu S et al. Neuroimage. 2005;28:401-409. 5. Just N et al. 2010.28:1143-1151. 6.Gruetter et al. MRM. 1993; 29:804. 7. Just N et al. Proc. 18th ISMRM scientific Meeting, Stockholm, Sweden, 1199. 8. Mlynarik V et al. MRM. 2006; 56:965. 9. Provencher SW. MRM. 1993; 30:672.**Acknowledgements:** Supported by the centre d'Imagerie Médicale (CIBM) of UNIL, EPFL, HUG, CHUV and Leenards and Jeantet foundations.