BOLD response and associated metabolic changes in the rat barrel cortex following sustained trigeminal nerve stimulation

N. Just[1,2], H. Frenkel[3], and R. Gruetter[3,4]
[1]LIFMET, CIBM, EPFL, Lausanne, Switzerland, [2]Department of Radiology, UNIL, Lausanne, Switzerland, [3]LIFMET, EPFL, Lausanne, Switzerland, [4]Department of Radiology, UNIL and HUG, Lausanne and Geneva, Switzerland

Introduction : The interest in investigating the dynamics of metabolite concentrations during sustained neuronal activity is due to the fact that previous studies both in rats and humans reported rather controversial results(1). 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 obtain measurable and reproducible sustained BOLD activation in the rat barrel cortex and moreover to evaluate the metabolite concentration changes in the rat barrel cortex during prolonged trigeminal nerve stimulation.

Materials and Methods: Male SD rats (n=6 for fMRI; n=3 for fMRS, 350±20g) were orally intubated and catheterized for α-chloralose (continuous intravenous infusion at 5mg/ml at a rate of 2ml/hour) and blood gas sampling every 30 minutes. The rat was positioned in a dedicated holder with head fixation and maintained under physiological conditions (pH ~7.4, pCO2~39-mmHg, MABP ~130mmHg, Temperature =37.5ºC ± 0.5ºC).**Trigeminal nerve stimulation:** Stainless steel electrodes were inserted in the hiatus infraorbitalis (2) and in the neck muscles respectively. Electrical stimulation was performed using an external stimulator (WPI, UK) and using the optimized stimulus parameters found previously (Stimulus pulse width=0.5ms, Stimulus frequency =1Hz, Stimulus current intensity=2mA)(3).**fMRI**: All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian) with 12cm gradients (400mT/m in 120µs). A quadrature Transmit/Receive 17mm surface coil was used. First and second order shims were adjusted using FASTMAP (4) (water linewidth of 13-15Hz in a 216µl volume). A single shot gradient echo EPI sequence (TR/TE=2500-2000/25ms; FOV=22x22mm; matrix=64x64; slice thickness = 0.5-1mm; 5 slices, BW=357 KHz) was used for image acquisition. The paradigm of stimulation was 1minOFF-30sON-1minOFF or 10minON-10min OFF-10min ON- or 1min OFF-5min ON-1min OFF repeated up to 30 minutes of acquisition. **fMRS:** Localized Proton spectroscopy was performed using SPECIAL(5) in a 9µl VOI localized in the activated barrel cortex and after adjusting once more the shims using FASTMAP. 35 blocks of 32 fids were acquired for a total acquisition time of 75 minutes. Sustained stimulation of the rat trigeminal nerve was performed according to the paradigm described earlier. **Data Analysis:**fMRI data were processed using STIMULATE (6). Cross correlation maps were calculated as in (3).All the data are presented as means ± S.E.M. Metabolite concentrations were calculated using LCmodel (7). Blocks of fids were summed over each 10 minute-period. The evolution of metabolite concentrations in the rat barrel cortex during a 10-minute sustained neuronal activity were thus compared to resting state concentrations measured in a similar manner. Statistics were performed using a paired-test. A pvalue <0.05 was considered significant.

Results: Fig1a shows an example of barrel cortex BOLD activation following sustained trigeminal nerve stimulation in three consecutive slices while 30 minute-time courses with 3 different paradigms applied during prolonged trigeminal nerve stimulation are shown in fig1b-c-d. Following the onset of 5 minute and 10 minute –long stimulation a strong overshoot over the signal can be observed that rapidly decays to a BOLD response corresponding to BOLD signal responses between 3.5 and 6%. Such results were obtained in 5 other rats in a similar manner. During the periods of stimulation, NAA and total Cr linewidths decrease on average by 0.4Hz. Fig2 shows examples of spectra obtained during a 10-minute rest period and during a 10-minute trigeminal nerve stimulation period respectively. Quantitative analysis of the spectral data show that mean Cramer-Rao lower bounds (CRLB) are under 30% for Asc, Cr, PCr, Gln, Glu, myo-Ins, NAA, PE and Tau during stimulation and rest. The mean CRLB for lactate was 40%±5%. Lactate levels increase from 0.71± 0.2µmol/g during rest to 1.06±0.3µmol/g (Mean ± SEM, n=3) during stimulation. Asc, myo-Ins and Tau concentrations show a tendency to decrease during stimulation whereas PCr/Cr concentrations increase.

Discussion/Conclusion: Sustained neuronal activation was performed both in rodents (1) and in humans (8). However, the BOLD activation time-course during prolonged stimulation has rarely been shown (9). Here, we demonstrate that trigeminal nerve stimulation is a suitable model to follow barrel cortex activation in the rat during 5 and 10 minutes of stimulation, reproducibly. The neurochemical profile of the rat barrel cortex during sustained stimulation was examined in 3 rats only. Several mean metabolite concentrations demonstrate alterations during neuronal activation but do not show statistically significant differences with resting states due to a large intra and inter-patient variability. In particular, a 33% mean increase in Lactate during stimulation is found that matches with previous findings by other groups (8,10,11).

References (1) Su X et al. Neuroimage, 28,401-409, 2005 (2) Nielsen and Lauritzen, J.Physiology, 2001, 773-85 (3) Just N.17th ISMRM, 2009,1603(4) Gruetter R et al. MRM,29:804,1993;(5) Mlynarik V et al. MRM, 56:965,2006 (6) Strupp JP, Neuroimage, 1996, 3, S607 (7) Provencher SW.MRM,30:672,1993 (8)Mangia S et al.JCBFM, 27,1055-1063,2007 (9) Hyder F et al, PNAS, 93,7612-7617,1996(10)Prichard L et al., PNAS, 88,5829-31,1991 (11) Frahm J et al, MRM,35,143-148,1996. **Acknowledgements:** Supported by the centre d'Imagerie Médicale (CIBM) of UNIL, EPFL, HUG, CHUV and Leenards and Jeantet foundations.

Fig1a

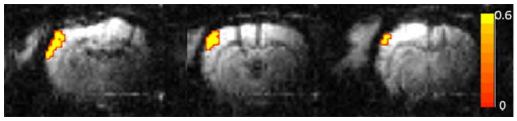

Figure1:a. Cross-correlation maps overlaid over single-shot GRE-EPI images showing BOLD activation in the barrel cortex following sustained trigeminal nerve stimulation and **b-c-d**. Examples of BOLD time course (% signal changes) following a b)1minOFF-30sON-1minOFF paradigm c) a 1min OFF-5minON-1minOFF paradigm and d)a 10minON-10minOFF-10minON paradigm. All 3 paradigms were repeated up to 30minutes of acquisition.**Figure2**: Example of spectra during a 10-minute resting state (no stimulation) and during a 10-minute trigeminal nerve stimulation