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LACTATE AND GLUTAMATE DYNAMICS DURING PROLONGED STIMULATION OF THE RAT BARREL CORTEX SUGGEST ADAPTATION OF CEREBRAL GLUCOSE AND OXYGEN METABOLISM

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Abstract—A better understanding of BOLD responses stems from a better characterization of the brain's ability to metabolize glucose and oxygen. Non-invasive techniques such as functional magnetic resonance spectroscopy (fMRS) have thus been developed allowing for the reproducible assessment of metabolic changes during barrel cortex (S1BF) activations in rats. The present study aimed at further exploring the role of neurotransmitters on local and temporal changes in vascular and metabolic function in S1BF. fMRS and fMRI data were acquired sequentially in α-chloralose anesthetized rats during 32-min rest and trigeminal nerve stimulation periods. During stimulation, concentrations of lactate (Lac) and glutamate (Glu) increased in S1BF by 0.23 ± 0.05 and 0.34 ± 0.05 μmol/g respectively in S1BF. Dynamic analysis of metabolite concentrations allowed estimating changes in cerebral metabolic rates of glucose (ΔCMR_{Glc}) and oxygen (ΔCMR_{O2}). Findings confirmed a prevalence of oxidative metabolism during prolonged S1BF activation. Habituation led to a significant BOLD magnitude decline as a function of time while both total ΔCMR_{Glc} and ΔCMR_{O2} remained constant revealing adaptation of glucose and oxygen metabolisms to support ongoing trigeminal nerve stimulation.

Key words: fMRS, BOLD, barrel cortex, glutamate, ΔCMRGlc, lactate, adaptation.

INTRODUCTION

As ^{1}H- and ^{13}C-functional magnetic resonance spectroscopy (fMRS) studies develop both in humans and animals, the use of prolonged paradigms of stimulation is increasing (Mangia et al., 2007; Schaller et al., 2014; Just et al., 2013; Sonnay et al., 2015, 2016) and therefore an increased understanding of the mechanisms underlying these prolonged activations is needed. In particular, adaptation mechanisms cause weakened BOLD responses to repeated stimuli and involve several effects such as fatigue, tuning changes and altered response dynamics (reviewed in Larsson et al., 2015). Adaptation to repeated stimuli has been characterized at different stages along the whisker to barrel cortex pathway and encompasses several definitions from adaptation of sensory receptors to enhancement of inhibitory phenomena. However, adaptation is an inherent factor that also enables discrimination of subtle differences between subsequently presented stimuli (Maravall et al., 2007; Adibi et al., 2013; Musall et al., 2014). In fact, investigation of adaptation mechanisms has shown to be a great source of information on cortical processing. Many studies both in humans and in rodents used these nonlinearities to distinguish neuronal populations or designed protocols to minimize them (reviewed in Larsson et al., 2015). Adaptation/habituation effects have been included in early models of the hemodynamic response to brain activation (Aubert and Costalat, 2002; Buxton et al., 2004) using functions describing a sharp BOLD increase followed by a plateau after several seconds or minutes of stimulation. To our knowledge, only a few neuroimaging studies have addressed the effects of adaptation *per se* (Vafaee et al., 2012; Mintun et al., 2002; Lin et al., 2009; Moradi and Buxton, 2013). These studies considered its impact on CMR_{O2} and CBF derived from PET or calibrated BOLD fMRI measurements since they may reflect changes in neurovascular coupling and suggested that investigating adaptation mechanisms during a prolonged stimulation process could be an interesting means of evaluating the balance between changes in CBF and changes in CMR_{O2} (Moradi and Buxton, 2013; Larsson et al., 2015). Interestingly, adaptation can reflect changes in neurovascular coupling without changing BOLD responses or neuronal activity (Moradi and Buxton, 2013; Enager et al., 2009). These latter effects, if not taken into account, may lead to misleading interpretation of neuroimaging signals (Moradi and Buxton, 2013).

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Abbreviations: fMRS, functional magnetic resonance spectroscopy; BOLD, Blood Oxygen Level Dependent; TGN, trigeminal nerve; S1BF, primary somatosensory barrel field cortex; Glu, glutamate concentration; GABA, γ-amminobutyric acid; Lac, lactate; Gln, glutamine; VOI, volume of interest; ΔCMR_{glc}, change in cerebral metabolic rate of glucose; ΔCMR_{O2}, change in cerebral metabolic rate of oxygen.

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Studies of the rodent whisker to barrel cortex pathway showed that understanding of neurovascular mechanisms cannot be dissociated from understanding neurometabolic signals (Enager et al., 2009). CMR_{O2} can be accurately measured using ^{13}C-MR spectroscopy of glucose metabolism requiring robust protocols and measurement techniques (Sonnay et al., 2016; Hyder et al., 2001). Nevertheless, models of energy metabolism can also be used to infer CMR_{O2} changes using ^{1}H-MR spectroscopy measurements of Lactate and Glutamate (Schaller et al., 2014) although a number of oversimplifications exist and their impact on the results are debatable: in particular in this model, lactate was assumed to reflect non-oxidative metabolism only whereas it can also reflect oxidative metabolism (Attwell and Iadecola, 2002) or changes in oxidative metabolism that are neglected in the absence of glutamate variations. Moreover, the number of ATP molecules accounted for the process was decreased assuming that not all the glucose is transformed.

Recently, we demonstrated that ^{1}H-fMRS studies could be translated to the rat barrel cortex (S1BF) during continuous prolonged activation (Just et al., 2013). Glutamate and lactate increased, as in human studies, further establishing these changes in metabolite concentrations as a hallmark of neuronal activity (Schaller et al., 2014).

In Just et al. (2013), continuous BOLD responses demonstrated strong habituation. We also measured prolonged BOLD responses to 2- hour forepaw stimulation in rats with a repeated 10sON-30sOFF-10sON paradigm of stimulation of the rat forepaw under α-chloralose anesthesia (Sonnay et al., 2015) and observed important BOLD baseline drifts that may be attributed to averaged local differences in CMR_{O2}, CBV and CBF across a large activated area. Habituation/adaptation phenomena in BOLD were minimized by switching stimulation frequencies between 2 and 3 Hz every 5 min. However, recent studies on adaptation demonstrated that BOLD responses do not accurately reflect adaptation processes (Buxton et al., 2014). Identical paradigms were used in a 13C-fMRS during infusion of glucose and 4-h forepaw stimulation of the rat forepaw (Sonnay et al., 2016). In these studies, we assumed that habituation effects had little impact on metabolic outcome.

The aim of this study was therefore to characterize and verify the presence and impact of habituation/adaptation processes determined with BOLD fMRI on the metabolic changes occurring during prolonged rat S1BF activation measured with an improved fMRS protocol including more rats and more averaging as well as a better positioning of the voxel of interest to avoid partial volume effects. Relative changes in CMR_{O2} and CMR_{Glc} were analyzed at 6.4 min after the start of a 32-min prolonged stimulation and after 12.8 min of prolonged stimulation to examine the effects of habituation.

EXPERIMENTAL PROCEDURES

Animals

All studies were performed following the approval of Service de la consommation et des affaires vétérinaires du canton de Vaud (Switzerland) and according to the federal guidelines of the Animal Care and approved by the local authority. Male Sprague–Dawley rats (n = 23, 350 ± 40 g; Charles River, L'Arbresle, France) under isoflurane anesthesia (2–3%) vaporized in 30% O_2 in air were intubated, and mechanically ventilated. Two femoral arteries and one femoral vein were catheterized for blood gas sampling and blood pressure measurements as well as α-chloralose (an initial intravenous dose of 80 mg/kg was administered followed by a continuous intravenous infusion of 27 mg/kg/h at a rate of 2 ml/h) and pancuronium administrations. Respiration rate was monitored through a pillow (SA Instruments, Stony Brook, NY, USA) placed underneath each rat. Temperature was measured using a rectal sensor and regulated via control of the temperature of water flowing through tubing covering the body of each rat and linked to a temperature-regulated bain-marie. Less than 300 μl of arterial blood was sampled every 30 min and blood parameters directly measured using an AVL blood gas analyzer (Dotmed, USA). Mean Arterial blood pressure (MABP) was measured continuously using a transducer attached to the femoral artery catheter. Body temperature and blood parameters were maintained at physiological levels (T = 37.5 °C ± 0.5 °C; pH = 7.4 ± 0.05, pCO2 = 39.7 ± 7 mmHg and MABP = 148.9 ± 11 mmHg) throughout each experiment. An intravenous femoral injection of Pancuronium Bromide (Sigma, Switzerland) of 0.7 ml per hour was performed to further minimize the remaining motion. Rats were positioned in a dedicated stereotactic holder equipped with ear and bite bars which was rotated in the magnet (30–45°) for a better positioning of voxels for fMRS over the barrel cortex and to avoid partial volume effects (Figs. 1 and 2).

Trigeminal nerve stimulation (TGN)

Electrodes were percutaneously inserted in the left infraorbital nerve. Electrical stimulation of the left trigeminal nerve (TGN) was performed using an external stimulator (WPI, Stevenage, UK) as described in (Just et al., 2010). The paradigm of stimulation for fMRI was 60sOFF-60sON preceded by one minute and a half before the start of the paradigm and the paradigm of stimulation for fMRS was 60sON-60sOFF repeated for 32 min with pulse duration of 0.5 ms, stimulation frequency of 1 Hz and stimulation current amplitude of 2 mA.

Magnetic resonance experiments

Experiments were performed on an actively shielded 9.4T/31 cm horizontal bore magnet (Magnex, Varian, Abingdon, UK) with 12-cm gradients (400mT in 120 μs) and an in-house made quadrature Transmit/Receive 20-mm surface coil. Shims were adjusted using FAST(EST)MAP (Gruetter and Tkác, 2000). fMRS was conducted first followed by BOLD fMRI in order to ensure that the metabolism was not perturbed by previous stimulation.