Mapping stimulus-evoked glutamate and lactate changes in the mouse brain using spectroscopic imaging

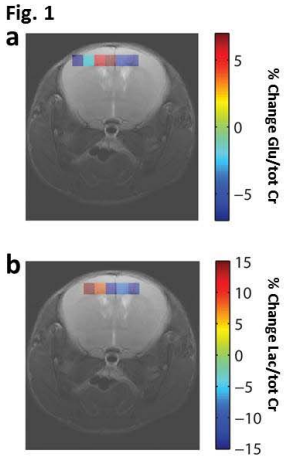
Aline Seuwen[1], Aileen Schröter[1], and Markus Rudin[1,2]

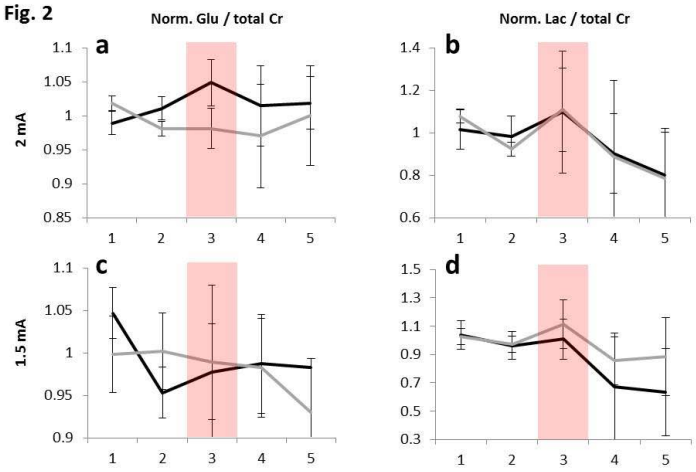
[1]*Institute for Biomedical Engineering, ETH & University of Zürich, Zürich, Switzerland,* [2]*Institute for Pharmacology and Toxicology, University of Zürich, Zürich, Switzerland*

Introduction Studying brain activity during sensory stimulation in mice is attractive in view of the numerous transgenic lines available only in this species. Conventionally, neuronal activity is accessed by evaluating activity-evoked local changes in blood oxygenation levels (BOLD contrast) using fMRI methods. In mice however, the BOLD signal seems to be dominated by systemic contributions [1]. Widespread and bilateral responses are generally observed following unilateral stimulation. ^{1}H-MRS (Proton Magnetic Resonance Spectroscopy) allows the quantification of several neurotransmitter and general indicators for brain metabolism [2] and could therefore constitute a valuable additional tool to study brain function and metabolism in mice, as changes in neurotransmitters are less prone to peripheral vascular confounds. Here, we attempted to visualize brain activation upon electrical paw stimulation in mice by quantifying changes in glutamate (Glu) levels, using spectroscopic imaging (SI). SI allows covering of extended brain regions, and hence monitoring of differential responses of brain areas, e.g. a direct comparison of activated versus non-activated regions. Our study aims to elucidate whether SI can serve to deliver a more specific readout on the activation of respective brain regions involved in processing of sensory stimuli in mice.

Method All experiments were carried out using a BioSpec 94/30 (Bruker BioSpin MRI GmbH, Ettlingen, Germany) small animal MR system operating at 400 MHz. A four-element receive-only cryogenic phased array coil (2x2 geometry, overall coil size 20x27mm2, Bruker BioSpin AG, Fällanden, Switzerland) was used in combination with a circularly polarized 86 volume resonator for transmission. All in vivo experiments were carried out in strict adherence with the Swiss law for animal protection. Mice (N=5 in each group) were anesthetized using isoflurane (1.25%); intubated and artificially ventilated with an oxygen/air (20% / 80%) mixture. An axial slice comprising somatosensory cortex, striatal regions and ventricles was selected using a 90° pulse, and immediately followed by the acquisition of the FID (acquisition delay: 1.298ms). For SI, following parameters were used: TR: 2500ms; FOV: 1.5x1.5cm; matrix: 17x17; slice thickness: 1.3mm; acquisition time: 12min. Scans were performed using VAPOR water suppression interleaved with six saturation slices for fat suppression. Field maps were used for shimming. The acquisition was weighted using a Hanning filter. SI scans were repeatedly acquired, alternating baseline and stimulation periods. Stimulation paradigms: 2 mA (N=5) and 1.5 mA (N=5), 5 Hz, 30s stimulation, 30s rest, 10 cycles (for a total duration of 10 min). Relative quantification was performed using LCModel [3].

Results Highly spatially resolved Glu and Lac maps acquired in 12 min allowed to separate signals from left and right somatosensory cortices (Fig. 1a,b). Increased Glu levels were measured during stimulation with both stimulus strengths (1.5 and 2 mA). Variability is still high (Fig.2) and optimization of the method is still in progress, however data collected so far shows higher Glu response in the somatosensory cortical area contralateral to the stimulated paw (2mA, Fig. 2a, 3a). Lac was increased as well, but differences between both hemispheres could not be observed (Fig. 2b,c). Quantified Glu level changes did not differ between the two stimulation strengths (Fig 3c).





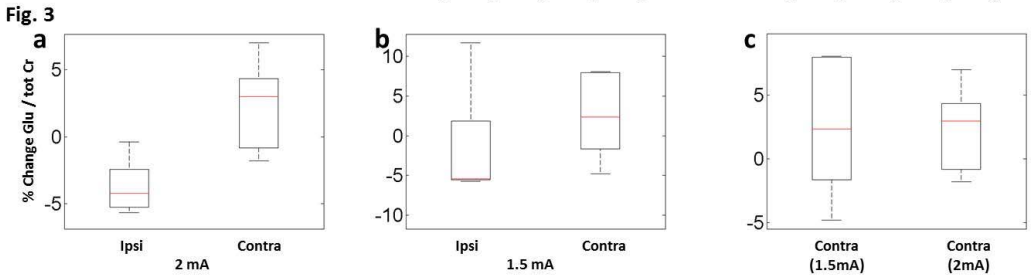


Fig. 1: Representative metabolite maps of Glu (a) and Lac (b) acquired in 12 min on the mouse brain. The values are expressed as percentual changes of the respective metabolite levels (relative to the baseline value) during electrical stimulation of the right hind paw (2 mA).

Fig. 2: Mean normalized changes of Glu (a, c) and Lac (b, d) levels during electrical stimulation in the right hind paw with 2 mA (a, b) and 1.5 mA (c, d) in a group of 5 animals. Left (black line) and right (grey line) somatosensory cortices are depicted. Stimulation was performed in the third time point, after two scans of baseline.

Fig. 3: Percentual change of Glu in the contralateral side to the stimulated paw compared to the ipsilateral side when stimulated with 2 mA (a) and 1.5 mA (b). No difference is observed between the two stimulation strengths (c)

Discussion As studying the processing of peripheral input using hemodynamic fMRI readouts in mice constitutes a major challenge, adapted paradigms and/or alternative fMRI readouts should be considered. Here we propose SI, and especially the measurement of changes in Glu levels as a complementary method to visualize brain activity. A tendential cortically lateralized change in Glu, a neurotransmitter associated with neuronal activation, demonstrates the feasibility and specificity of such approach. However, so far the technique still suffers from low sensitivity (i.e. high stimulus strengths are required to trigger a response). Further efforts in optimizing measurement technique should eventually also decrease inter-trial variability.

References [1] Schroeter et al., Neuroimage 94, 372–384. [2] Duarte et al., Neuroimage 61, 342–362 [3] Provencher S.W., NMR Biomed. 14, 260–264.