Functional spectroscopic imaging: mapping glutamate levels in the mouse brain during electrical stimulation of the hind paw

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Introduction Glutamate is the major excitatory neurotransmitter in the brain. Therefore changes in glutamate levels can be associated with changes in neuronal activity. ^{1}H-MR spectroscopy allows measuring brain metabolism *in vivo*, and has been applied to monitor changes in glutamate concentration accompanying functional brain activation in a single voxel [1]. Recently, we have applied slice selective FID acquisition of proton spectroscopic imaging (FIDLOVS, [2]) in mice, to monitor metabolic changes upon pharmacological stimulation using the GABAA antagonist bicuculline [3]. High signal intensity resulting from the FID acquisition combined with the superior sensitivity provided by a cryogenic phased array coil enabled serial spectroscopic imaging (SI) acquisitions with a temporal resolution of 12min and voxel dimensions of 1 ul. This temporal resolution has been sufficient to visualize functional changes in GABA, glutamate, glutamine and lactate levels during the administration of the neuroactive compound. In the present work, the same method was applied to monitor focal changes in glutamate concentration in the mouse somatosensory cortex triggered by electrical stimulation of the hind paw. The study was prompted by our recent observation [4], that BOLD fMRI responses to the same stimulation paradigm revealed widespread signal changes comprising bilateral brain areas: it was concluded that systemic contributions dominated the specific stimulus evoked response in the somatosensory cortex. The objective of the current study was to evaluate whether an alternative readout of brain activity such as changes in local glutamate levels would provide superior regional specificity.

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) was used in combination with a linearly polarized room temperature volume resonator for transmission. The cryogenic array coil was provided by Bruker BioSpin AG, Fällanden, Switzerland. All in vivo experiments were carried out in strict adherence with the Swiss law for animal protection. Mice (N=3) were anesthetized using medetomedine (bolus of 0.1mg/kg, with subsequent infusion of 0.2mg/kg/h (i.v.)); intubated and artificially ventilated with an oxygen/air (20% / 80%) mixture. An axial slice 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 paradigm: 2mA, 5 Hz, 30s stimulation, 30s rest, 10 cycles (for a total duration of 10 min). Relative quantification was performed using LCModel [5].

Results SI data obtained in 12 min with 1 µl voxel dimensions enabled the quantification of glutamate (Glu, 2.35ppm) in several voxels located in left and right somatosensory cortexes (Fig. 1a). Additional metabolites, such as glutamine (Gln), taurine (Tau), GABA, and myo-inositon (Ins) could be identified in the spectra (fig.1.b), but were not further analyzed in the context of this study. Glu levels (expressed as ratio Glu/Cr+PCr) were extracted from 4 to 5 voxels comprising both somatosensory cortices (2 voxels were averaged for each side) and plotted as a function of time (fig.2 a,b). A distinct increase in glutamate has been observed in the contralateral side during electrical stimulation, while glutamate levels remained almost constant in the ipsilateral side. In the first stimulation period (right hind paw), glutamate was found to be increased by 8±1.2% in the left somatosensory cortex (1% on the right side), while it was increased by 11±7.2% in the right somatosensory cortex during stimulation of the left hind paw (Fig 3). The Cramer-Rao lower bounds (CRLB, LCModel) were estimated around 4% on average in the cortex (Fig.3).

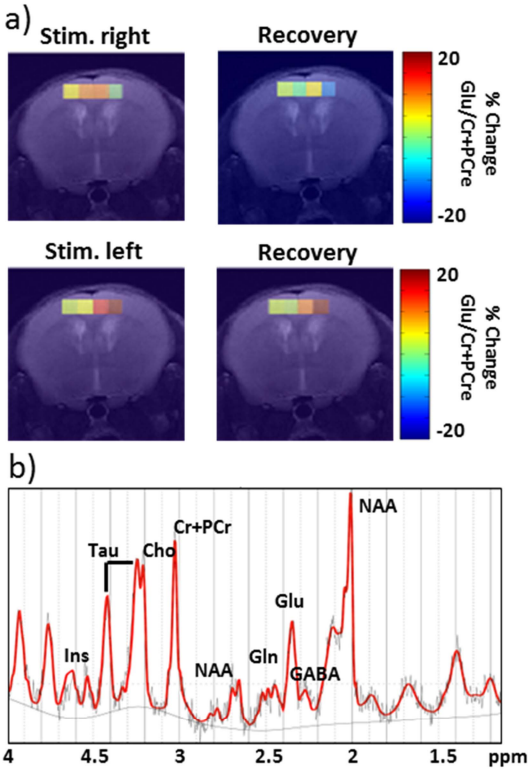

Fig 1: SI data in the mouse brain. The resolution was sufficient to quantify individual voxels for left and right somatosensory cortices (a). Glutamate (2.35 ppm) could be reliably quantified (b).

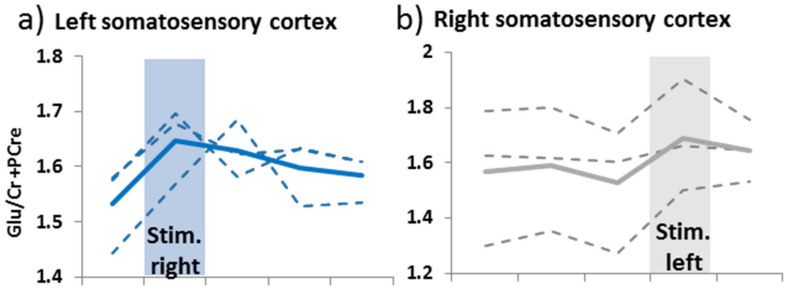

Fig 2: Glutamate time course upon electrical stimulation in the hind paw. Curves from 3 animals (dashed lines) are extracted from the right (a) and left (b) somatosensory cortex. Individual animals and the average (solid line) show a clear increase in Glutamate levels during stimulation.

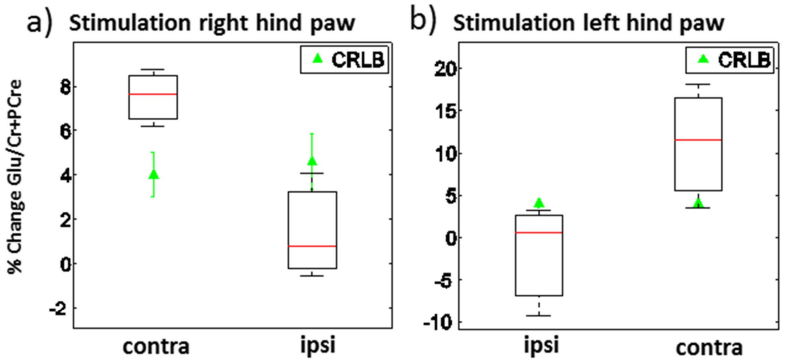

Fig 3: Percentage of Glutamate changes, contralateral side versus ipsilateral side. Percentage changes were found to be much higher in the contralateral somatosensory cortex for both stimulation periods, displaying the expected unilateral response.

Discussion & outlook SI has been successfully applied to monitor glutamate changes over extended regions in mouse brain elicited by electrical stimulation with a spatial resolution of 1ul voxels. Unilateral stimulation of the hindpaws led to significant changes in glutamate levels by approximately 10% in the contralateral somatosensory cortices while the corresponding ipsilateral region remained unaffected. This is in contrast to our observations using BOLD fMRI using an similar electrical stimulation protocol, which revealed a widespread bilateral response. The neurotransmitter response appears to be more specific, i.e. it is less susceptible to confounding peripheral hemodynamic effects. Measuring glutamate level in a temporo-spatially resolved manner constitutes an attractive readout of neuronal activity complementing conventional BOLD-fMRI. Based on the data quality, further acceleration of the SI protocol appears conceivable.

References [1] Xu S, Yang J, Li C. Q, Zhu W, Shen J, NeuroImage 28 (2005) 401 – 409 [2] Henning A, Fuchs A, Murdoch JB, Boesiger P. NMR Biomed. 2009;22(7):683-96. [3] Seuwen A, Schröter A, Rudin M, Proc. Intl. Soc. Mag. Reson. Med. 21 (2013) [4] Schröter A, Schlegel F, Seuwen A, Grandjean J, Rudin M, Proc. Intl. Soc. Mag. Reson. Med. 21 (2013) [5] Provencher SW. Magn. Reson. Med. 1993; 30(6):672–679.