Local glial energy metabolism supports glutamatergic neurotransmission during increased focal cortical activity: a ^{13}C MRS study in the rat cortex *in vivo*

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Target Audience: Scientists with interest on brain energy metabolism.

Purpose: Brain energy metabolism results from cellular cooperation between neurons and astrocytes [1-3]. Although both cell types regulate synaptic transmission via the glutamate-glutamine cycle, the relevance of glial pathways coupled to neuronal metabolism during brain activity is still unclear [1,3]. The aim of the study was to measure the contribution of glial and neuronal metabolic fluxes by direct ^{13}C MRS *in vivo* during prolonged cortical activation.

Materials and Methods: Sprague Dawley rats (n=15, 299±16 g) under α-chloralose anesthesia were stereotaxically fixed and positioned in a homebuilt holder. Electrical stimulation was achieved through stainless steel electrodes inserted between the digits of both hind- and forepaws. Square pulses (0.5 ms width) were delivered at constant current (2.5 and 3 mA for the fore- and hindpaws, respectively) and variable frequency (2-3 Hz). The stimulation paradigm was 30 s ON - 10 s OFF repeated for 4 h, switching the frequency every 5 minutes. Cortical activation was confirmed by blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) using gradient echo echo planar imaging (GE-EPI). MRS experiments were performed on a 14.1 T/26 cm horizontal bore magnet with a homebuilt coil consisting of a ^{1}H quadrature surface coil combined to a ^{13}C linearly polarized surface coil. After FAST(EST)MAP shimming, localized ^{1}H and ^{13}C spectra (during infusion of [1,6-^{13}C]glucose) were acquired from a 93.5 µL volume with STEAM and semi-adiabatic distortionless enhancement by polarization transfer (DEPT) combined with 3D-ISIS for ^{1}H localization [5], respectively. LCModel was used for analysis of both ^{1}H and ^{13}C spectra [6]. The scaling of ^{13}C fractional enrichment (FE) curves was based on MRS of brain extracts [3]. Data was fitted to a two-compartment model and variance of parameters was determined by Monte-Carlo analyses [3].

Results: Prolonged BOLD response following the applied paradigm was detected over 4 h in the cortex prior ^{13}C MRS experiments (fig.1A). The relative activated volume confirmed by BOLD fMRI was large enough to detect FE curves of carbon position C4, C3 and C2 of glutamate and glutamine, and carbon position C3 and C2 of aspartate (fig.1B). Rats under stimulated brain activity (n=7) showed faster neuronal oxidative metabolism compared to rest (n=8) (fig.1C). More precisely, intermittent focal brain activity resulted in a significant increase in the neuronal transmitochondrial exchange rate V_x^n (+47%), while smaller increases in neurotransmission rate (V_{NT} +33%), neuronal and glial TCA cycle rates (+7% V_{TCA}^n and +17% V_{TCA}^g, respectively), glutamine synthetase rate (V_{GS}, +24%), glial transmitochondrial exchange rate (V_x^g +22%), and cerebral metabolic rate of glucose oxidation ($CMR_{glc(ox)}$, +10%) were observed.

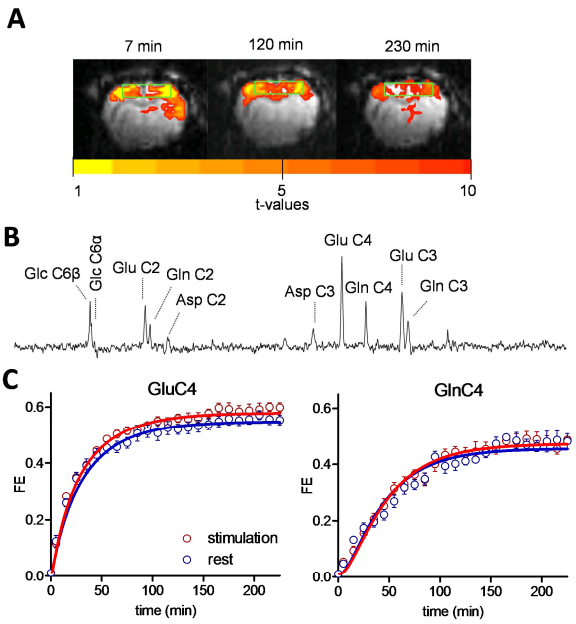

Figure 1: (A) Representative t-value map showing activation of cortex overlaid on a GE-EPI image after 7 min, 120 min, 230 min of stimulation. The color scale represents t-values. (B) Typical *in vivo* ^{13}C MRS spectrum acquired at 14.1 T in a 93.5-µl volume during the last 30 min of [1, 6-^{13}C]glucose infusion. (C) FE curve of carbon position C4 of glutamate and glutamine. The red and blue lines represent the best fit for the stimulation and the resting group, respectively.

Discussion**:** Data from the brain of stimulated rats and at rest indicate that the neuron-glia interactions adapt to different brain activity states in such a way that focal cortical activity likely increases neuronal and glial oxidative metabolism, supporting increased neurotransmission rates. The small changes are likely related to the high ubiquitous, baseline neuronal activity associated to α-chloralose anesthesia [4].

Conclusion: Energy metabolism in astrocytes responds to increased brain activity, as in neurons, with increased mitochondrial oxidation to produce energy for neurotransmission support, namely glutamate clearance and conversion to electrophysiologically inactive glutamine.

References: [1] Hyder *et al.* (1996) PNAS 93(15):7612. [2] Gruetter *et al.* (2001) Am J Physiol, 281(1):E100. [3] Duarte *et al.* (2011) Front Neuroenergetics 3:3. [4] Shulman and Rothman (2014) JCBFM 34(11):1721. [5] Henry *et al.* (2003) MRM 50:684. [6] Henry *et al.* (2003) NMR Biomed 16:400.

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