Investigating Brain Energetics During and After Rat Whole Brain Occlusion

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INTRODUCTION *In vivo* magnetic resonance spectroscopy (MRS) is a powerful tool to investigate brain energetics during whole-brain occlusion. Simultaneously acquired ^{1}H and ^{31}P spectra provide lactate, phosphocreatine (PCr), ATP, and pH dynamics. To correlate these metabolic results with neuronal responses, the occlusion model was investigated using electrophysiology, with a focus on local field potential (LFP) frequencies. Interestingly, the MRS and electrophysiology show distinctly different temporal responses, revealing complex correlations between the metabolites and neuronal activity. Using the information gained from these acquisition methods, we seek to understand the underlying metabolic mechanisms of brain ischemia.

METHOD *Rat model and MRS Acquisition*: An acute 4-blood-vessel-occlusion (4BVO) model[1] was applied in Sprague-Dawley rats (n = 13) to induce transient whole brain occlusion(s) under 2.0% isoflurane. The severe 12-minute occlusions (1-2 per rat) were monitored with MRS or electrophysiology. In one group, multi-nuclei ^{1}H/^{31}P MRS[2] was acquired during occlusion and recovery at a 9.4T/31 cm horizontal-bore animal magnet (Agilent, CA) with a localized ^{1}H (PRESS, TE = 20 ms) and global ^{31}P pulse sequence, TR = 4s. In a second group, 32-channel linear array electrodes (NeuroNexus, MI) were implanted bilaterally in somatosensory cortex. Local field potential (LFP) signals were collected at 2 kHz (Blackrock Microsystems, UT). All methods followed the guidelines of the National Institutes of Health and were approved by the IACUC of the University of Minnesota. *Data Processing*: Post-processing and analysis was performed in MATLAB (MathWorks, MA). Phased MR spectra were used for identifying and integrating metabolic peaks, and calculating metabolite concentrations. Baseline ^{1}H spectra were subtracted from the occlusion and recovery spectra to minimize macromolecule contamination in lactate integration. Group averaged ^{31}P spectra were used for pH calculations[3] and ATP integration. LFP signal (0-200 Hz) was filtered from continuous electrophysiology recordings. Power spectrograms were calculated and averaged across 4 LFP frequency bands: Low (LF, <10 Hz), Medium (MF, 10-30 Hz), Low-Gamma (LG, 30-60 Hz), and High-Gamma (HG, 60-200 Hz).

RESULTS & DISCUSSION Occlusion induced changes in lactate, PCr, ATP, and pH (Fig 1A) were investigated using the multi-nuclei ^{1}H/^{31}P MRS. The dynamic changes of Δ[PCr] were found to be consistent across occlusions and animals, allowing spectral averaging to increase SNR for the quantification of ATP and pH. Group pH dropped from 7.1 at baseline to 6.2 before reperfusion. The rate of pH decrease closely matched that of lactate, and continued to match during reperfusion until a buffer capacity was reached. [ATP] remained at baseline for roughly 1.5 minutes after occlusion onset before following the same depletion pattern as [PCr]. To quantify response rates, exponential fits were applied to the [PCr] and [lactate] trends. [PCr] occlusion and recovery rates were similar, and were driven by metabolism. The rate of Δ[lactate] during occlusion was significantly faster than the recovery. The fast lactate production is due to lack of ATP, while the [lactate] recovery is likely dominated by slow washout. When correlating lactate vs. PCr changes, the data splits into 2 distinct loop trends (Fig 2), highlighted by different maximum [lactate] changes.

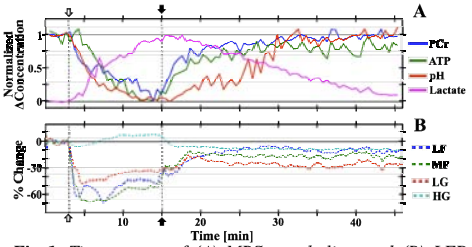

Fig 1. *Time course of (A) MRS metabolites and (B) LFP band power during occlusion and reperfusion.*

Maximal changes in brain lactate concentration were estimated from literature values[4,5] based on a simplified metabolism model of a closed, oxygen-deficient brain system. Glucose was estimated at 2 mM in the isoflurane anesthetized brain tissue and 0.5 mM in brain blood considering 5% blood volume fraction, while glycogen was estimated at 4 mM. At 2 lactate per glucose or glycogen, an increase of 13 mM lactate was calculated. The data from the big-loop trend (Fig 2B) supports this estimation with a maximum Δ[lactate] of 13.9 mM. The small-loop trend could indicate reduced energy reserves, possibly due to poor recovery from surgery or animal physiological condition.

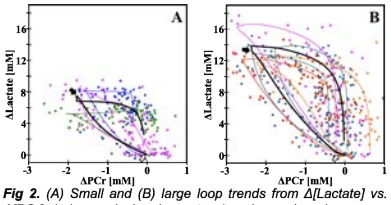

Fig 2. *(A) Small and (B) large loop trends from Δ[Lactate] vs. Δ[PCr] during occlusion (open to closed arrow) and recovery. Black: average trend, Color: individual rats.*

A representative electrophysiology channel spectrogram (Fig 3A) was used for preliminary comparison with MRS results. The burst-suppression state of the rat is obvious in the LFP trace (Fig 3B), and allowed for easy visualization of brain activity changes. Baseline bursts occurred at ~0.2 Hz and lasted 1-2 seconds. Power within four frequency bands was averaged to allow comparison of temporal trends (Fig 1B). Bursts, LF, MF, and LG were reduced within 30 seconds of occlusion onset. The lack of significant power reduction in HG suggests that low frequencies might be more dependent on a stable energy pool than high frequencies. While the continuous trace remained isoelectric in appearance, HG power increased during the second half of the occlusion in some channels. Other bands showed a less pronounced, but similar power increase across the occlusion. Reperfusion resulted in a brief depression of power, then a slow recovery in low frequencies. Power levels in all bands recovered to a new steady-state approximately 10 minutes post-occlusion. However, LFP bursts remained absent for 10-15 minutes following reperfusion, with bursts not approaching baseline rates until 30+ minutes post-occlusion. Pre-occlusion baseline levels for power bands or bursting were not reached.

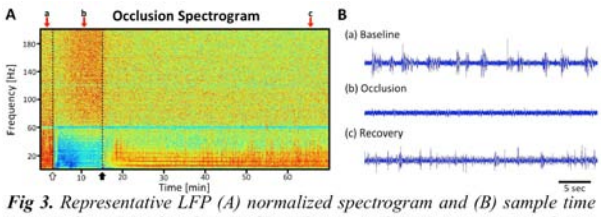

Fig 3. *Representative LFP (A) normalized spectrogram and (B) sample time courses from (B-a) baseline, (B-b) occlusion, and (B-c) recovery are shown, highlighting changes in bursting.*

LFP and ATP recoveries appear to correlate best (Fig 1), as the original baseline levels were never achieved, with a particularly high correlation between ATP and LG Dynamics of lactate, PCr, and pH do not appear to highly correlate with any of the LFP bands, hinting at differing or complex underlying metabolic mechanics, perhaps caused by a delayed effect on the metabolic response.

CONCLUSION Comparison of MRS and electrophysiological responses to whole-brain occlusion in rat provide insight into interesting correlations between brain energetics and neuronal activity. Most markedly, LFP power appears to begin to recover during occlusion, despite a lack of bursts and the continual decrease of ATP and PCr. For reperfusion, lactate and PCr recover to baseline values, while ATP and LFP do not. Low frequency LFP appears to be more affected by the energy deficiency than high frequency LFP. Further investigation will look at the reproducibility of these correlations and trends across cortex regions and animals, with the goal of increased understanding of the effects of transient occlusion on the rat brain.

ACKNOWLEDGEMENTS This work is supported in part by NIH grants NS057560, NS041262, NS070839, P41 RR008079 & EB015894, P30 NS057091 & NS5076408, and the Keck Foundation.

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