Investigating Longitudinal Metabolite Changes Associated with Epileptogenesis in vivo in a Rat Model of Interictal Spiking Using ^{1}H MRS at 7 Tesla

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Purpose: Most animal model studies of epilepsy have yet to examine longitudinal changes associated with persistent interictal spiking activity, as detected by intracranial EEG recordings, in the neocortex. We believe that interictal spikes play a key role in the formation of epileptic seizures, as evidenced by similar gene activation patterns found in our tetanus toxin rat model of interictal spiking as well as those found from surgically excised human epileptic neocortex[1]. Our rat model provides an ideal platform to study the effect of interictal spikes and epileptogenesis because it is a chronic , spontaneous *in vivo* model, with late onset seizures and minimal neuronal loss that mimics many hallmark features observed in human epilepsy patients[2]. Additionally, this animal model allows us to longitudinally examine interictal spiking activity as well as metabolite changes over time, including N-acetylaspartate (NAA), creatine plus phosphocreatine (Cr+PCr), glycerylphosphorylcholine plus phosphorylcholine (GPC+PCh), glutamate and myo-inositol using *in vivo* ^{1}H MRS at 7T. Some of these metabolites may be associated with epileptogenesis and the epileptogenic zone. The identification of these key markers will help guide future clinical approaches in epilepsy management and drug development.

Methods: In our preliminary study, two groups of Sprague-Dawley rats were studied: surgical sham control group (N=5) and treatment group receiving a single 1 µl tetanus toxin injection at a concentration of 10-25 ng/µl into the left somatosensory cortex (AP -1 mm, L 3.5 mm relative to bregma) (N=7). All rats had 4 MRI-compatible silver EEG recording screw electrodes implanted (2 on each hemisphere; AP +4 mm, -1 mm, L 3.5 mm relative to bregma) and a single reference electrode located above the nasal sinus. Electrodes were secured using dental cement and connectors were exteriorized to the back using back-mounting adaptors (Plastics One Inc.). All EEG recordings were made using a Stellate Harmonie recording system with a 200 Hz sampling rate, every other day for 2 hours.

A total of 4 ^{1}H MRS sessions per animal were performed every other week, starting with an initial baseline measurement prior to surgery. All measurements were done on a 7T Bruker ClinScan with a Siemens console using a 2 channel phased array receive only surface coil. The animals were anesthetized using isoflurane and shimming was performed locally with FASTESTMAP. Single voxel ^{1}H MRS of both water suppressed and unsuppressed signals were acquired in 4 locations (Figure 1), using PRESS (TE=14 ms, TR=3,500 ms, 256 averages, BW=3,500 Hz, 2048 points, 3.0 x 3.2 x 2.0 mm^3 voxel dimensions). Initial baseline scans were done for each rat approximately 5 days prior to surgery. Quantitation of ^{1}H MRS results were done using LCModel.

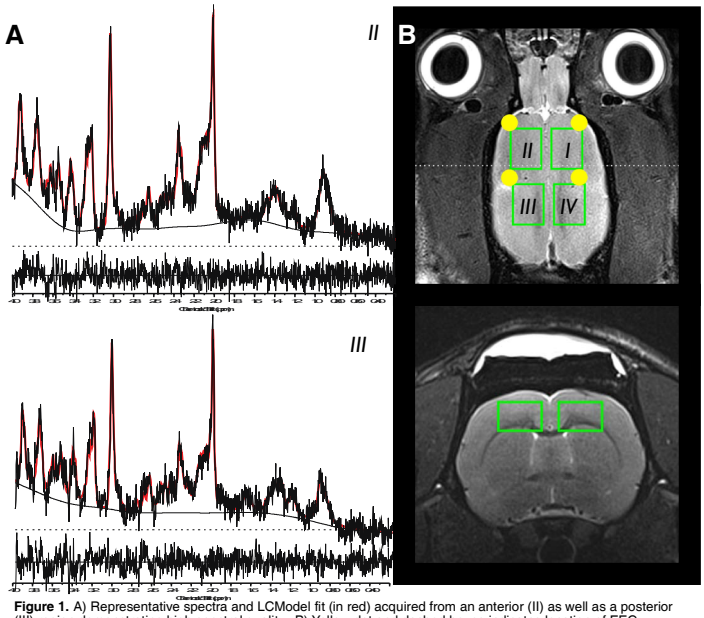

Figure 1. A) Representative spectra and LCModel fit (in red) acquired from an anterior (II) as well as a posterior (III) region demonstrating high spectral quality. B) Yellow dot and dashed boxes indicates location of EEG recording electrodes and green boxes indicate size and placement of MRS voxels.

Statistical analysis tested for temporal changes in metabolite levels in response to treatment with tetanus toxin when compared to controls using repeated measures generalized linear model (i.e. SAS PROC GENMOD). Model included metabolite levels as dependent variable and treatment (tetanus or sham control), time point (1, 2, 3, 4), hemisphere (left or right), and treatment by time point interaction as main effect terms. Two types of analysis were done using the described model; one treated time point as a continuous variable to examine differences in trajectory between tetanus and control (Analysis 1) and the other treated time point as a categorical variable to enable a post hoc time point by time point comparison of metabolite differences between tetanus and control groups using least square means analysis (Analysis 2). Anterior voxels (regions I & II) and posterior voxels (regions III & IV) were analyzed independently using both types of analysis.

Results: Our preliminary results using Analysis 1 indicate a significant time point-by-treatment interaction reflecting decreasing glutamate levels with increasing time point in the tetanus treated animals when compared to controls in the posterior cortical regions ($p < 0.05$). In the anterior cortical regions, we observe a notable interaction between treatment and time point for GPC+PCh ($p = 0.10$) as well as creatine plus phosphocreatine (Cr+PCr) ($p = 0.16$), reflecting an increase in both of those metabolites over time.

While the interaction between time point and treatment from Analysis 2 failed to showed significant differences between the tetanus treated and control groups ($p > 0.05$), an examination of the post hoc least square means analysis showed patterns consistent with those observed in Analysis 1. The posterior cortical regions show significant decrease in glutamate ($p < 0.01$) and myo-inositol ($p < 0.05$) within the tetanus treated group relative to the control group approximately 5 weeks post tetanus toxin injection (i.e., at time point 4). We also observed a significant relative increase in Cr+PCr ($p < 0.001$) amongst the tetanus treated group at time point 3. The anterior cortical regions similarly show a relative increase in Cr+PCr ($p < 0.05$) and GPC+PCh ($p < 0.05$) at time point 3. Additionally, post hoc analysis of anterior cortical regions also indicates a relative increase in glutatmate ($p < 0.10$) amongst the tetanus treated group for both time points 2 and 3 (approximately weeks 1 and 3 post tetanus toxin injection).

Conclusion: This work provides a first and preliminary look into the longitudinal changes in neocortical metabolite levels in a chronic animal model of epilepsy and we anticipate many of these non-significant observations will reach statistical significance as more animals complete the study. Most frequently reported metabolite change associated with epilepsy is a decrease in NAA levels, although most of these studies were confined to the hippocampus. Our examination of the cortical regions do not implicate changes in NAA, but instead point to potential region dependent changes in cell signaling and neuronal excitability. The differential changes in glutamate levels within the anterior and posterior voxels may reflect the relative function of the various regions encapsulated by the voxels. In general, the anterior voxel locations correspond to mostly motor and somatosensory cortices while the posterior voxel locations correspond to multiple regions including secondary visual cortex, medial parietal association cortex, along with portions of motor and somatosensory cortices and hippocampus. Additionally, regional changes in synaptic architecture as well as cellular energy dynamics are indicated by the relative increase in GPC+PCh and Cr+PCr respectively, indicating possible reorganization, especially at 5 weeks post tetanus toxin injection. Future correlations with EEG in this model will give additional insight into how these metabolites will change in relation to electrophysiology.

1. Barkmeier, D. T., Senador, D., Leclercq, K. *et al. Neurobiol. Dis.* 1–10 (2012).
2. Beaumont, T. L., Yao, B., Shah, A. *et al. J. Neurosci.* **32,** 14389–14401 (2012).