Investigating Longitudinal Metabolite and Electrophysiologic 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), GABA, and glutamate, 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.

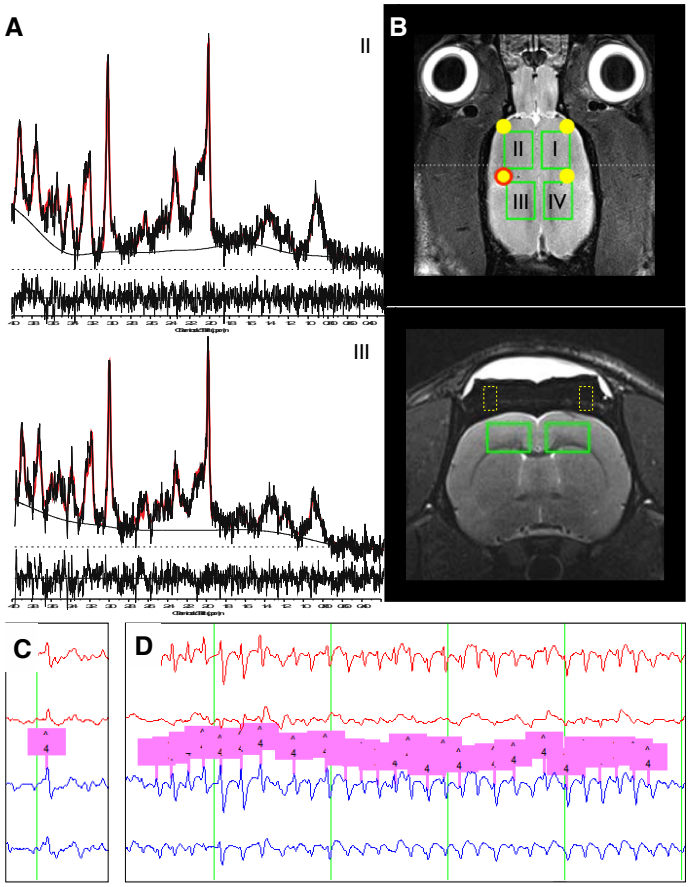

Figure 1. A) Representative spectra and LCModel fit (in red) acquired from an anterior (II) as well as a posterior (III) regions demonstrating high spectral quality. **B**) Yellow dot and dashed boxes indicate EEG recording electrode locations; red circle indicates tetanus toxin injection site; green boxes indicate size and placement of MRS voxels. **C**) Recorded rat EEG demonstrating an isolated interictal spike along with **D**) almost 5 second train of spikes.

Methods: In our study, 4 month old male Sprague-Dawley rats were treated with 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; Figure 1b) 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 to 3 hours.

A minimum of 4 ^{1}H MRS sessions per animal (maximum of 6) was performed every other week, starting with an initial baseline measurement prior to surgery. A total of 4 regions per animal were measured per scan. The regions consisted of two anterior left and right hemisphere quadrants (regions II and I on Figure 1b, respectively) as well as two posterior left and right hemisphere quadrants (regions III and IV on Figure 1b, respectively). 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 all 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. LCModel was used to quantify raw ^{1}H MRS spectra.

We tested for temporal changes in metabolite levels in response to treatment with tetanus toxin in conjunction with changes in interictal spiking frequency, as determined from intracranial video EEG recordings. Statistical testing was done using repeated measures mixed linear model (i.e. SAS PROC MIXED). Model included metabolite levels as dependent variable, spiking frequency and time point (1 through 6) as main effect terms along with spiking frequency by time point as the interaction term. A significant frequency by time point interaction term would indicate spiking frequency's ability to influence metabolite levels, over the time.

Results: Our results indicate regionally specific changes in metabolite levels in response to interictal spiking frequency, as moderated by the progression of time. Specifically, we found the interaction of spiking frequency and time point to be significant for NAA in the left anterior quadrant ($p = 0.011$), for glutamate, GABA, and lactate in the right posterior quadrant ($p = 0.003$, $p = 0.027$, $p = 0.006$, respectively). Regarding directionality, both NAA and lactate had negative effect estimates for the interaction, indicating that as the animals progressed in time, spiking frequency had a decreased effect on the levels of NAA and lactate. When examining the effect of time alone, NAA demonstrates a significant increase over time ($p < 0.05$) in the left anterior quadrant while no change in lactate was detected ($p > 0.05$) in right posterior quadrant. On the other hand, in the case of GABA and glutamate, the interaction effect estimates were positive, indicating that spiking frequency had a diminishing effect on the levels of GABA and glutamate. Neither GABA nor glutamate demonstrated significant changes over time when examining the effect of time alone ($p > 0.05$, both).

Conclusion: This work provides a first look into the longitudinal changes in neocortical metabolite levels in a chronic animal model and the interplay between interictal spikes and metabolite levels over time. Most frequently reported metabolite change associated with epilepsy is a decrease in NAA levels, although most of these studies were confined to the hippocampus. However, in our studies, we observe a significant increase in NAA over time in a region confined to the rat cortex. This, taken together with spiking frequency's apparent lack of ability to influence NAA levels over time points to possible neuronal synaptic changes and reorganization in response to the application of tetanus toxin. Changes reflected by GABA and glutamate in response to spiking frequency over time would indicate alterations in neurotransmitter balance, resulting in increased tissue excitability. Further studies are warranted to gain additional understanding of the precise mechanisms underlying these temporally dynamic metabolite and electrophysiologic changes.

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).