Sleep Deprivation and c-Fos Studies in Epileptic Mice

Jacob Kovalchek, Eastern Michigan University
Mackenzie Palmer, Whitman College
Kalume Lab

Seattle Children’s
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UW Neurological Surgery Summer Student Program
Our Project

Aim 1
Examine the effect of sleep deprivation on epileptic activity in a genetic mouse model of focal cortical dysplasia (FCD)

Aim 2
Examine seizure-induced neuronal activity in the hippocampus of a mouse model of Dravet Syndrome (DS) using c-Fos immunoreactivity
Our Project

Aim 1
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Examine seizure-induced neuronal activity in the hippocampus of a mouse model of Dravet Syndrome (DS) using c-Fos immunoreactivity
FCD in Humans and Mice

- FCD: cortical malformation resulting in intractable seizures
- Mouse model of FCD was generated by knock-in of a Pik3ca gain-of-function mutation (Millen Lab, CIBR)
- Recapitulates most of human FCD phenotypes, including epileptiform EEG activity and larger neurons

Aim 1

Seizures are rare in FCD mice

Kalume, unpublished
Method 1: Total Sleep Deprivation

- 5 hours SD beginning at 9AM
- 1 hour EEG and video recording following SD
- Control: sham experiment
Method 2: Data Collection

Interictal spike

Seizure

Myoclonus

Scale: 100uV/1s
Increased incidence of epileptiform activity after SD

**Aim 1**

![Graph showing increased interictal spikes after sleep deprivation compared to baseline.](image-url)
Increased incidence of epileptiform activity after SD

Aim 1
Increased incidence of epileptiform activity after SD
Conclusion (Aim 1)

Sleep deprivation increases the frequency of interictal spikes, myoclonia, and seizures in FCD mice.
Our Project

Aim 1
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Aim 2
Examine seizure-induced neuronal activity in the hippocampus of a mouse model of Dravet Syndrome (DS) using c-Fos immunoreactivity
Dravet Syndrome in Humans and in Mice

• DS: severe, childhood-onset epilepsy caused by heterozygous loss-of-function mutation in the SCN1A gene
• Mouse model of DS
  – Originally generated in the Catterall Lab (University of Washington)
  – Knockout of Scn1a
  – Recapitulates main phenotypic traits of DS, including high susceptibility to temperature-induced seizures

Aim 2

Brain regions involved?
Method: Immunocytochemistry

c-Fos antigen, a marker for neuronal activity

Fluorescent tags on c-Fos-specific antibodies mark c-Fos expression

Aim 2
Methods

Temperature-induced seizure

30 min

Perfused and fixed brain tissue with PFA; brain extracted and sliced

2 days

Slices stained to highlight c-Fos expression

Slices imaged using Zeiss LSM 710 microscope

Aim 2
# Experimental Groups

<table>
<thead>
<tr>
<th>Shorthand</th>
<th>Genotype</th>
<th>SCN1a Mutation</th>
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</thead>
<tbody>
<tr>
<td>Global Knockout (KO)</td>
<td>Global Scn1a knockout</td>
<td>Heterozygous in all cells</td>
</tr>
<tr>
<td>Control/Wildtype</td>
<td>Global Wildtype</td>
<td>No mutation (control)</td>
</tr>
<tr>
<td>Interneuron Knockout (KO)</td>
<td>Het Scn1a flox/Dlx56cre+</td>
<td>Heterozygous in forebrain interneurons</td>
</tr>
<tr>
<td>Control/Wildtype</td>
<td>Het Scn1a flox/Dlx56cre-</td>
<td>No mutation (control)</td>
</tr>
</tbody>
</table>

Aim 2 n=9
c-Fos Analysis: Seizure vs. No Seizure in Global KO

Fig. 1 c-Fos+ counts for global KO mice, compared across seizure conditions (insufficient sample size for t-test)
c-Fos Analysis: Seizure vs. No Seizure in Interneuron KO

Fig. 2 c-Fos+ counts for interneuron KO mice, compared across seizure conditions (p=0.31; p>0.05)
c-Fos Analysis: Post seizure across genotypes

**Fig. 3** c-Fos+ counts for mice that had seizures, compared across genotypes (insufficient sample size for ANOVA test)

Aim 2

http://mouse.brain-map.org/
Conclusions (Aim 2)

- Post-seizure c-Fos+ labeling in hippocampal dentate gyrus:
  - Increased in Global KO mice
  - Decreased in Interneuron KO mice
  - Higher in Global KO mice as compared to Interneuron KO mice
Future c-Fos Study

- Increased sample size
- c-Fos+ expression at multiple timepoints
- Different brain regions
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