Sleep Deprivation and c-Fos Studies in Epileptic Mice

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Center for Integrative Brain Research

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

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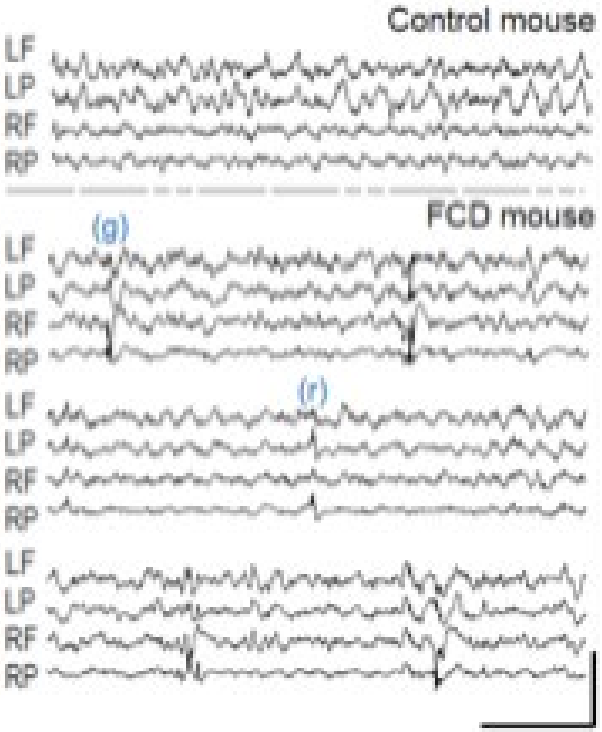
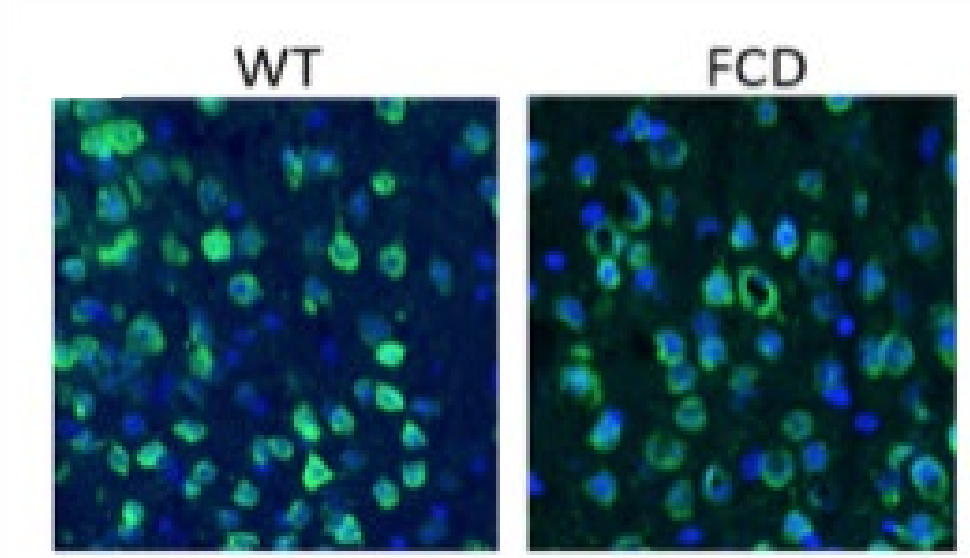
FCD in Humans and Mice

- 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



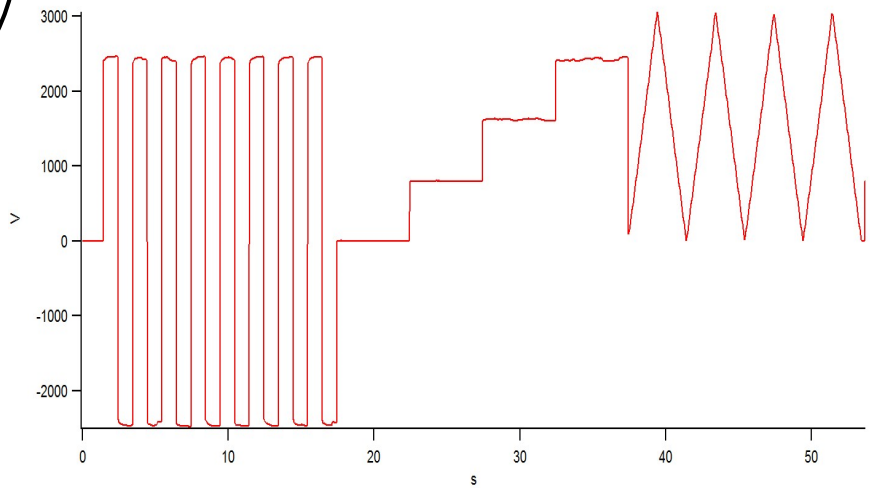


Seizures are rare in FCD mice

Kalume, unpublished

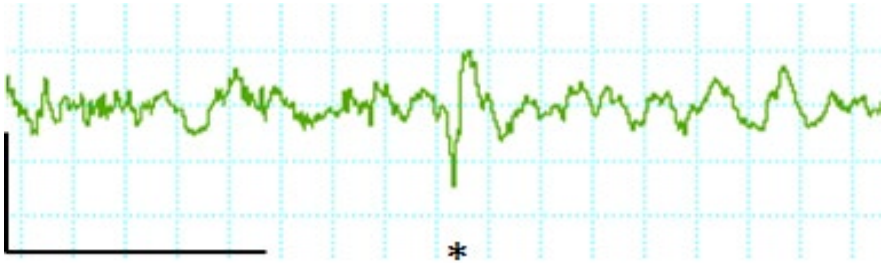
Method 1: Total Sleep Deprivation

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

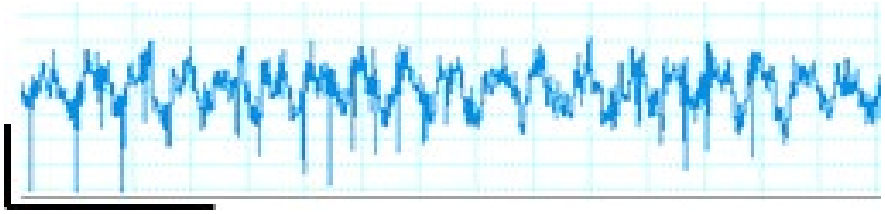


<http://www.adinstruments.com/products/powerlab>

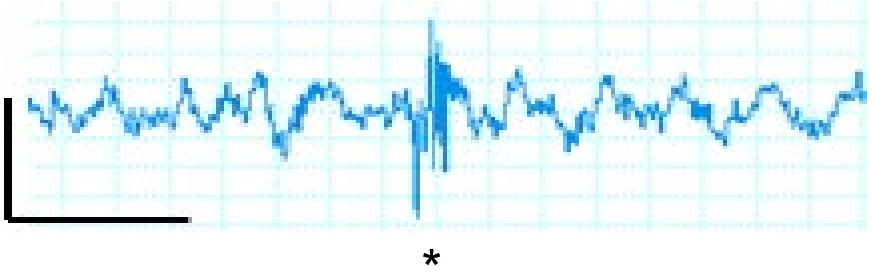
Method 2: Data Collection



Interictal spike

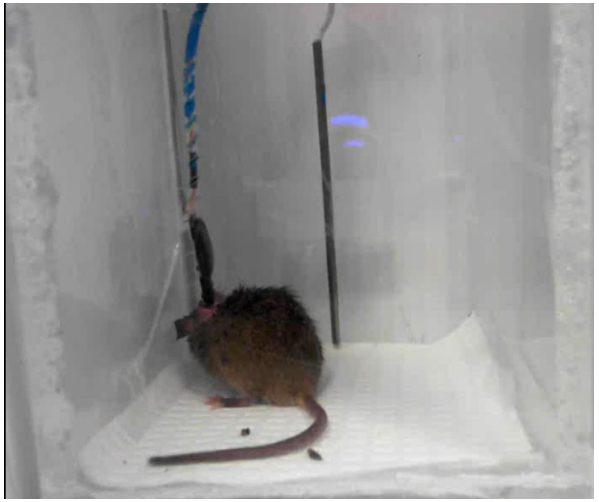


Seizure

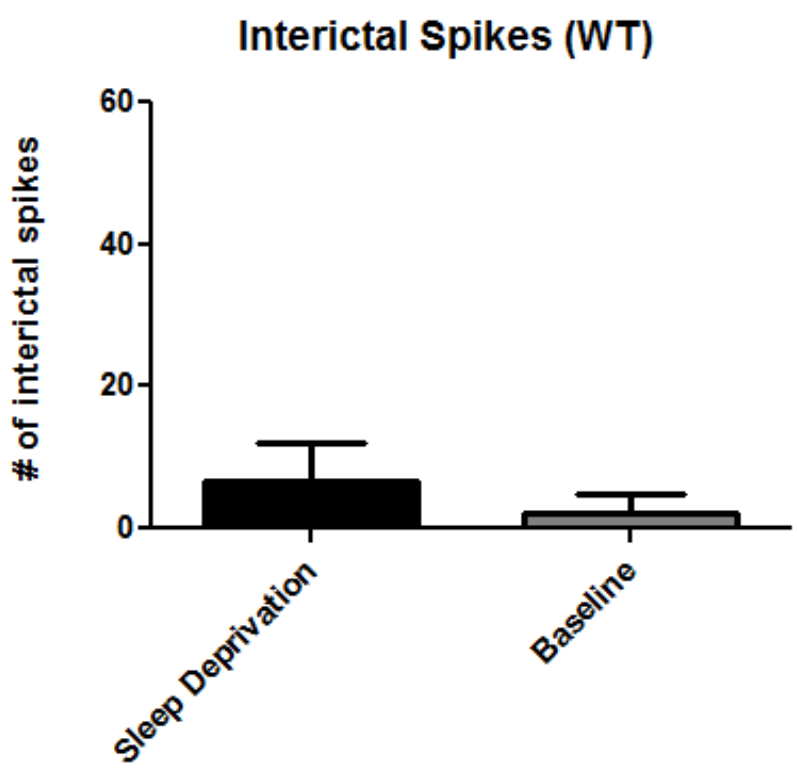


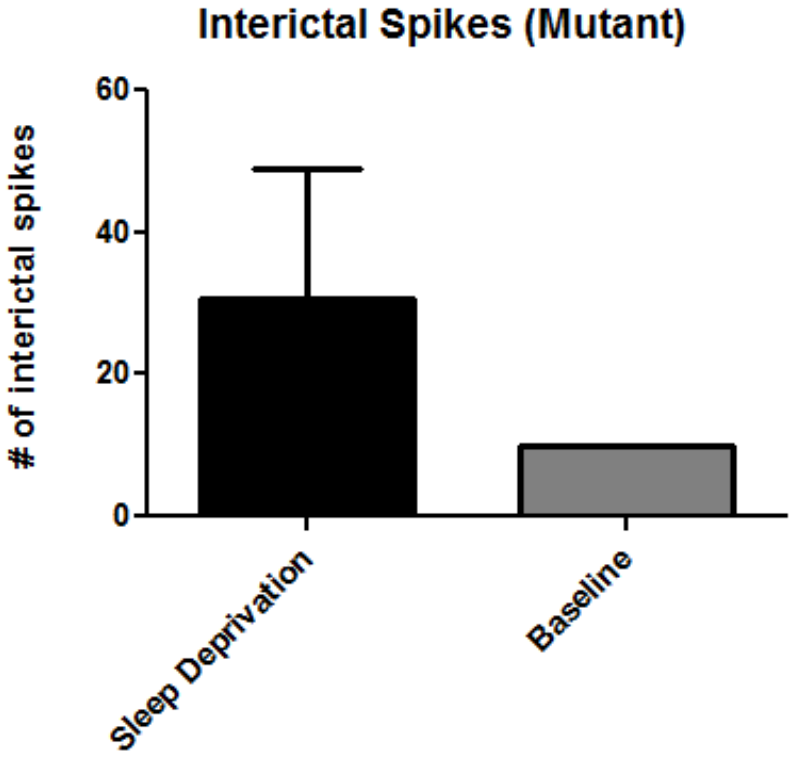
Myoclonus

Scale: 100uV/1s



Increased incidence of epileptiform activity after SD





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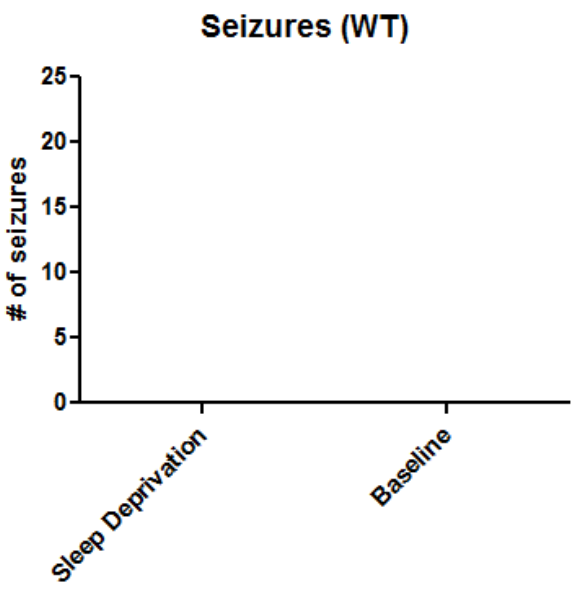
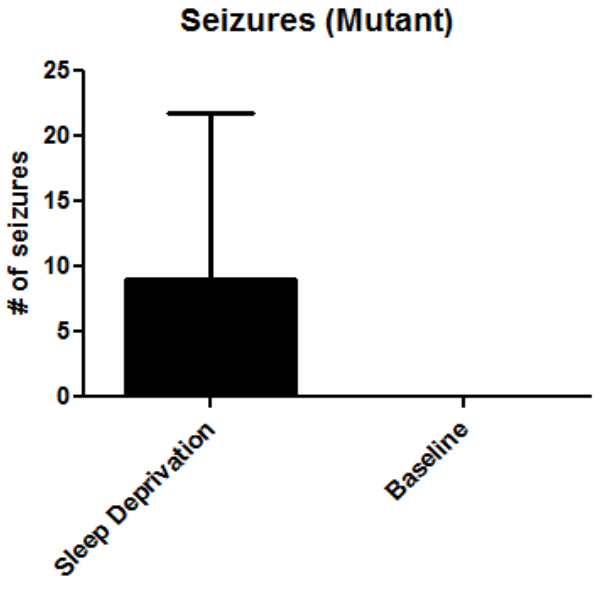
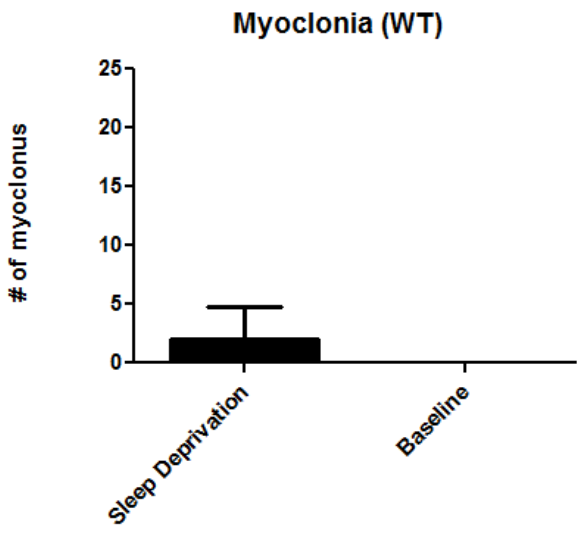
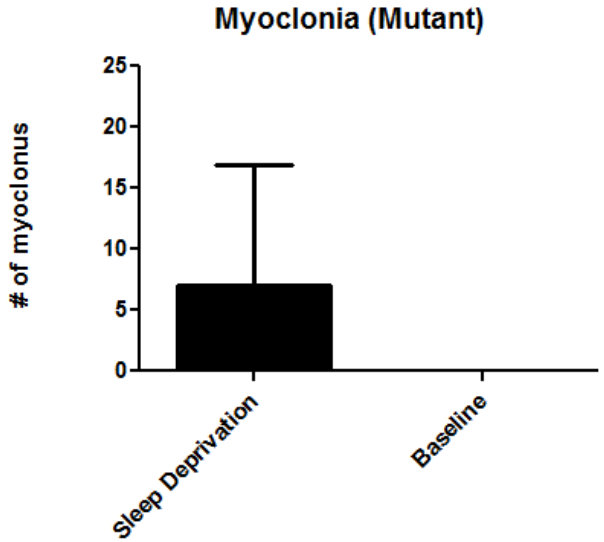
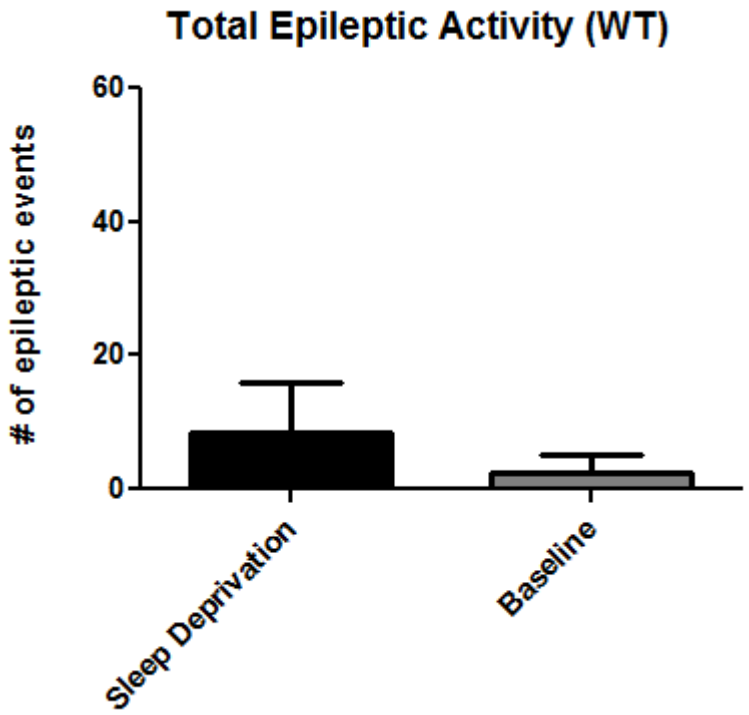


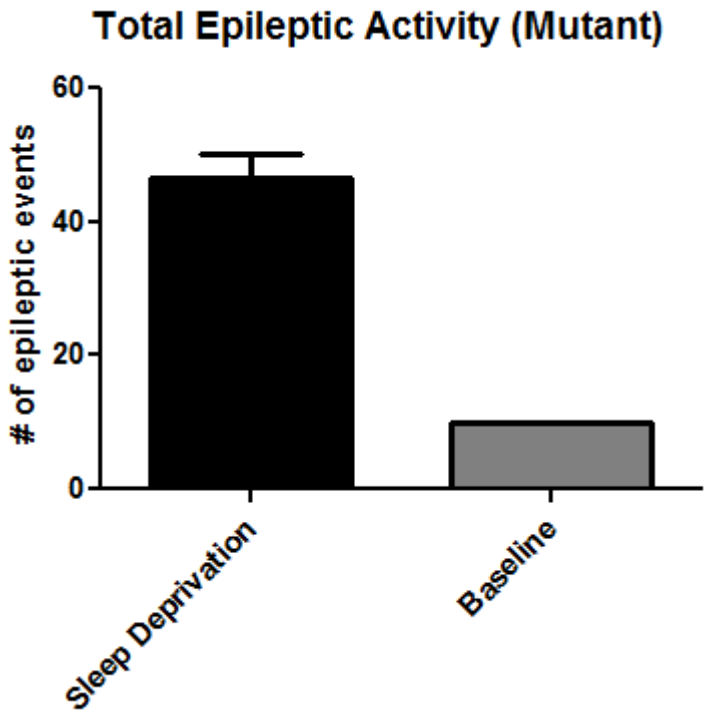






Increased incidence of epileptiform activity after SD





Conclusion (Aim 1)

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

Our Project

Aim 1

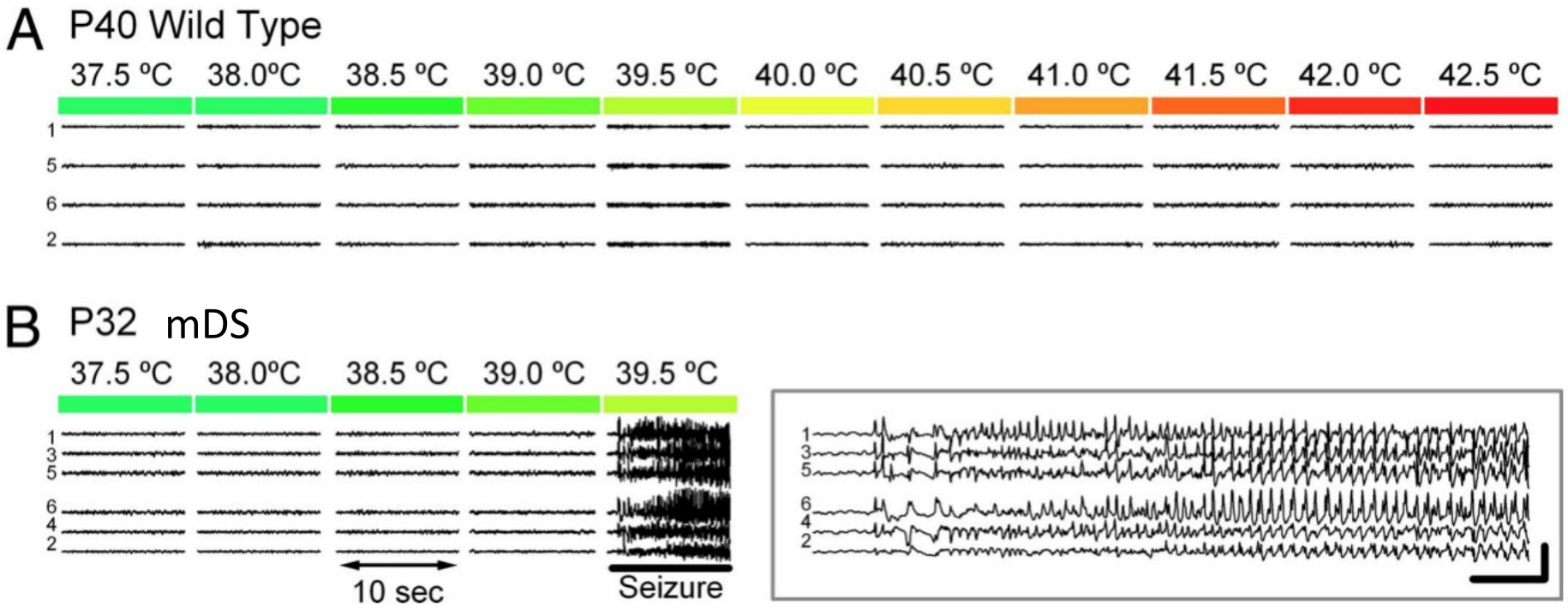
Examine the effect of sleep deprivation on epileptic activity in a genetic mouse model of focal cortical dysplasia (FCD)

Aim 2

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

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

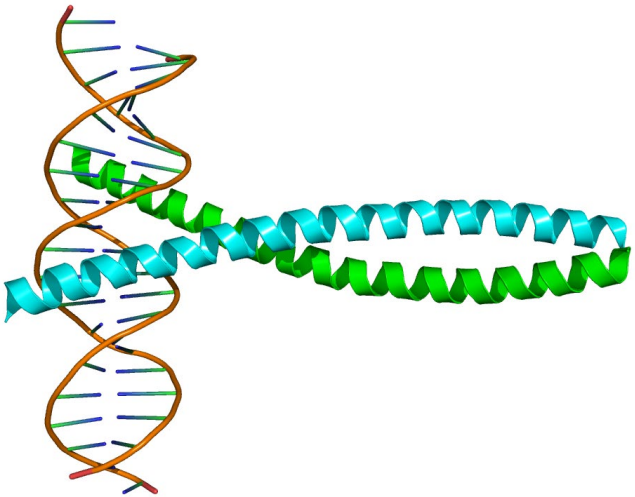


Aim 2

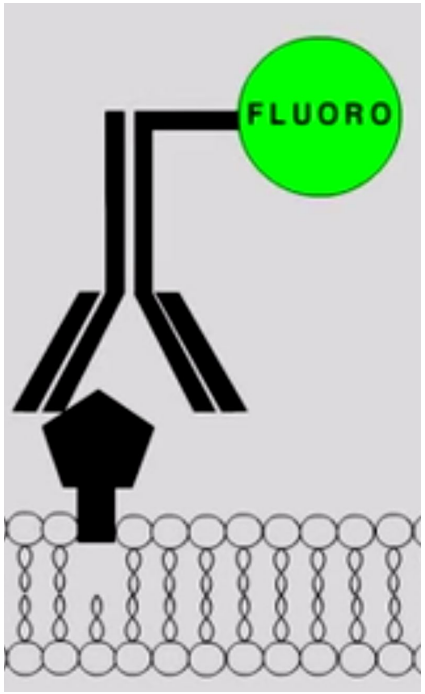
Brain regions involved?

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2656193/

Method: Immunocytochemistry



c-Fos antigen, a marker for neuronal activity

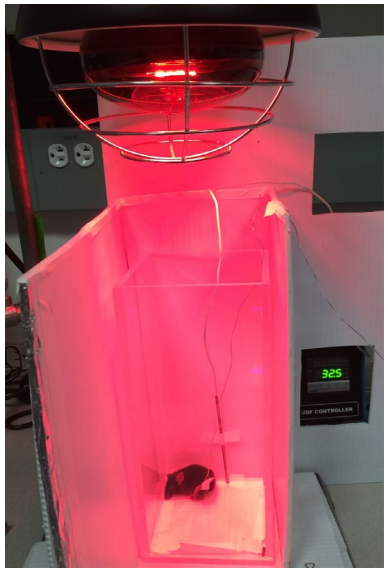


Fluorescent tags on c-Fos-specific antibodies mark c-Fos expression

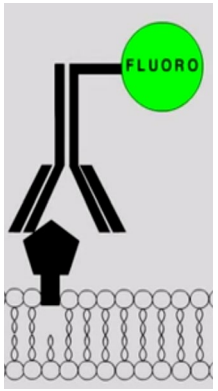
https://en.wikipedia.org/wiki/AP-1_transcription_factor

https://www.youtube.com/watch?v=ir8QzfADa4w

Methods



Temperature-induced seizure

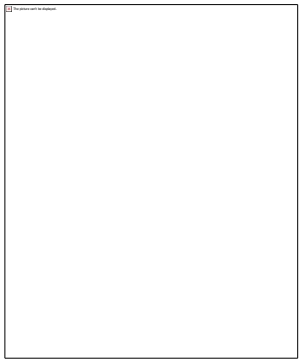


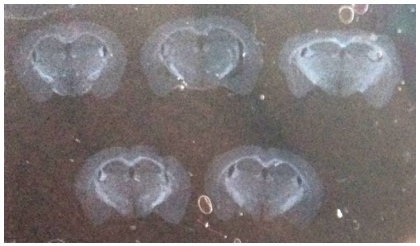
Slices stained to highlight c-Fos expression

30 min

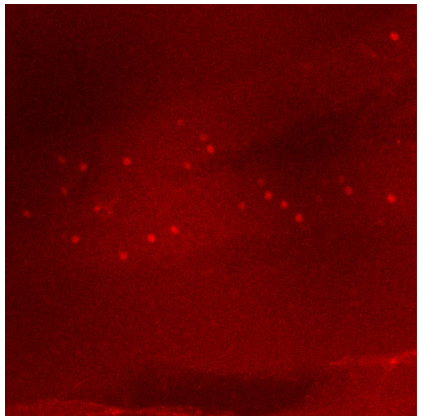
2 days

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





Slices imaged using Zeiss LSM 710 microscope



Aim 2

http://www.physitemp.com/support/TCAT-2DF%20PAK%20400%20Operating%20Manual.pdf

https://www.youtube.com/watch?v=ir8QzfADa4w

Experimental Groups

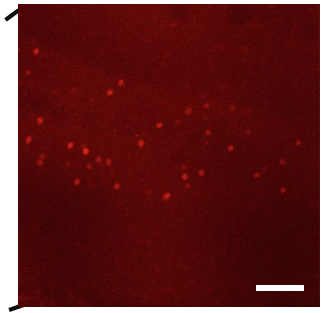
Shorthand	**Genotype**	**SCN1a Mutation**
Global Knockout (KO)	Global Scn1a knockout	Heterozygous in all cells
Control/Wildtype	Global Wildtype	No mutation (control)
Interneuron Knockout (KO)	Het Scn1a flox/Dlx56cre+	Heterozygous in forebrain interneurons
Control/Wildtype	Het Scn1a flox/Dlx56cre-	No mutation (control)

Aim 2

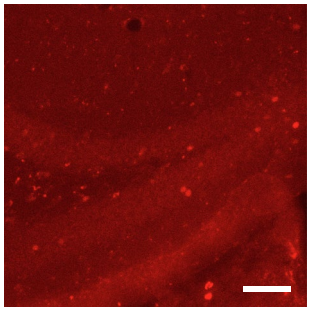
n=9

c-Fos Analysis: Seizure vs. No Seizure in Global KO

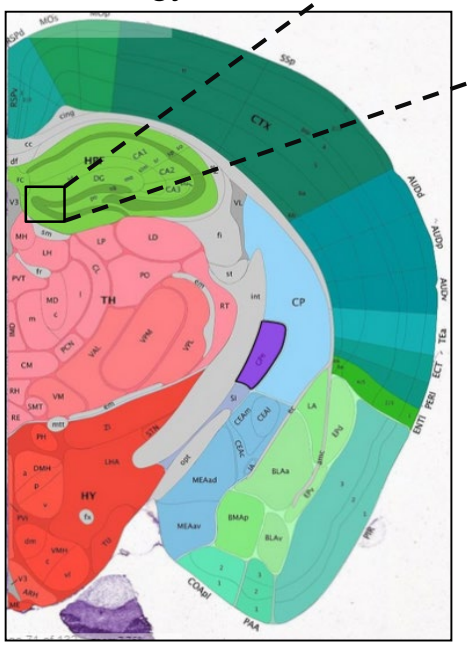
Seizure



No Seizure



Dentate gyrus



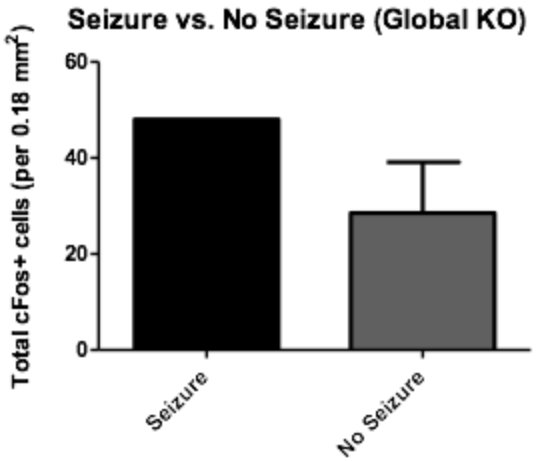


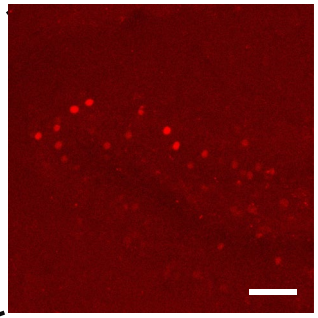
Fig. 1 c-Fos+ counts for global KO mice, compared across seizure conditions (insufficient sample size for t-test)

Aim 2

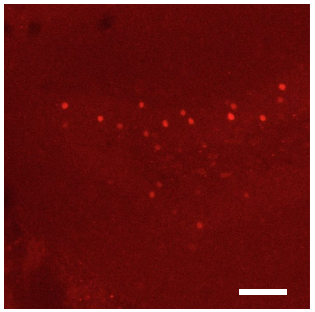
http://mouse.brain-map.org/

c-Fos Analysis: Seizure vs. No Seizure in Interneuron KO

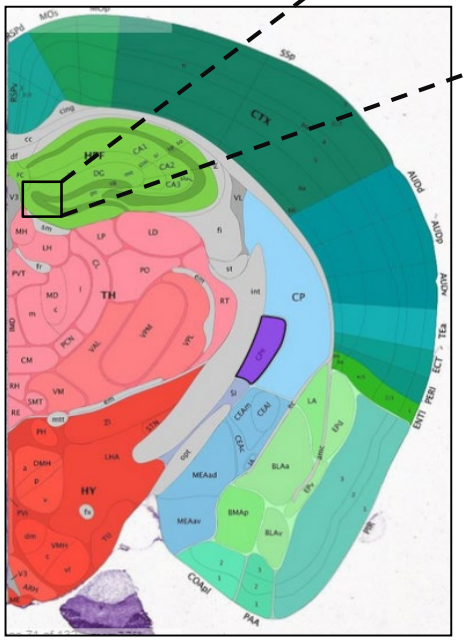
Seizure



No Seizure



Dentate gyrus



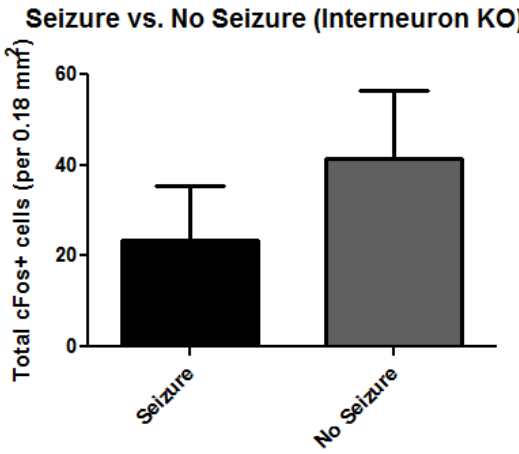


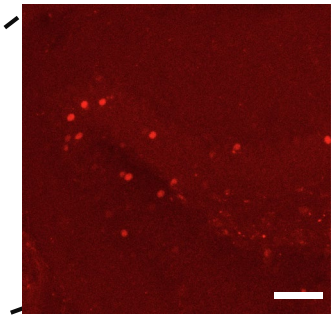
Fig. 2 c-Fos+ counts for interneuron KO mice, compared across seizure conditions ($p=0.31$; $p>0.05$)

Aim 2

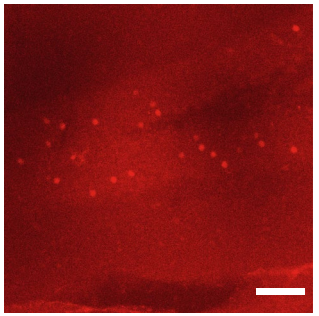
http://mouse.brain-map.org/

c-Fos Analysis: Post seizure across genotypes

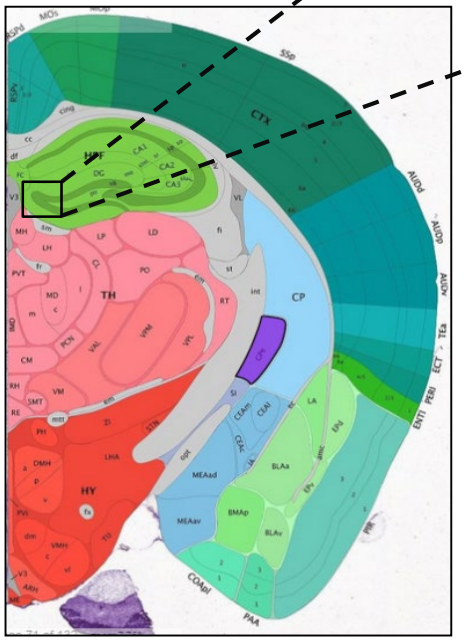
Wildtype



Global KO



Dentate gyrus



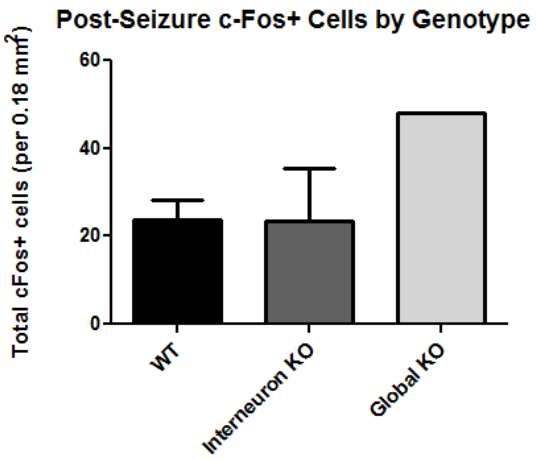


Fig. 3 c-Fos+ counts for mice that had seizures, compared across genotypes (insufficient sample size for ANOVA test)

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

Acknowledgments

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  - Andrew, Nikhil, Michelle, Curtis, Alexa, Vy
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http://www.hydroassoc.org/docs/2015WALK/NinoBiography.pdf