MicroRNA-mediated modulation of electrographic activity in a *Cntnap2* mouse model of epilepsy

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What is *Cntnap2* and why study epilepsy?

- *Cntnap2* encodes contactin-associated protein-like 2 (CASPR2), a protein that plays a vital role in neuron-glia interaction and action potential propagation **(Poliak et al., 2000)**
- *CNTNAP2* mutations in humans are associated with epilepsy and autism **(Friedman et al., 2008)**, and display symptoms including seizures and intellectual disability **(Strauss et al., 2006)**
- 5 million people are diagnosed with epilepsy every year **(WHO, 2019)**, and annual costs of epilepsy on our society are about $28 billion **(Prescott et al., 2020)**

Mouse model:

- *Cntnap2* knockout mice are a vital tool to study this gene's association with epilepsy, as first reported by ***Peñagarikano et al., 2011***
  - The *Cntnap2* model of epilepsy displays seizures, abnormal EEG patterns, and neuronal migration **(Thomas et al., 2016)**

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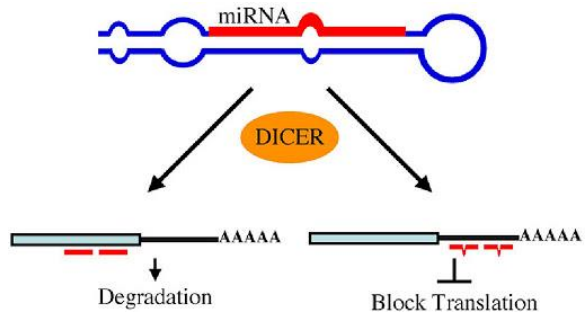
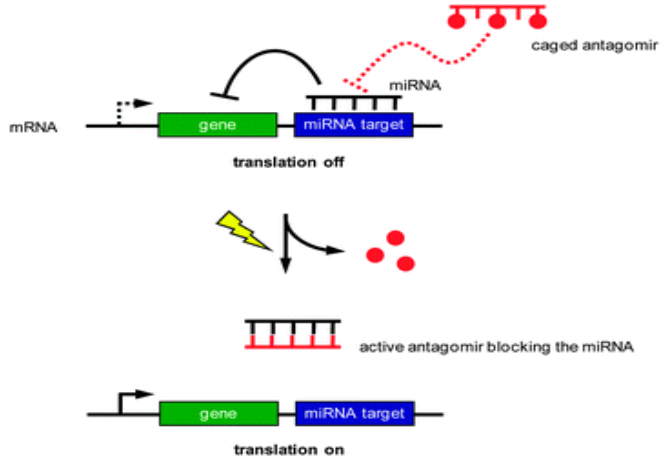
What are microRNAs (miRNA)?

- MiRNAs are non-coding single stranded RNAs which regulate post-transcriptional expression of mRNA and can be targeted to regulate gene expression through translational inhibition



- MiRNAs play a vital role in regulating seizures in epilepsy.
  - Based on previous work in the Gross Lab and other studies, two notable miRNAs are vital for role in regulating neuronal network and providing neuroprotection
    - miR-324-5p **(Gross et al., 2016); (Tiwari et al., 2019)**
    - miR-218-5p **(Kaalund et al., 2014)**

- **Antagomir Treatments as a therapy for epilepsy**
  - Antagomirs are antisense oligonucleotide treatments targeted to miRNA sequences which can regulate the expression of specific proteins by preventing the binding of miRNA to their mRNA targets
  - They have been shown to have therapeutic effects in the regulation of epilepsy **(Gross et al., 2016); (Tiwari et al., 2019)**



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Aim

- **Research Question:**
  - Does the inhibition of candidate mircoRNAs miR-218-5p and miR-324-5p using antagomirs affect seizure susceptibility in the *Cntnap2* knockout mouse model of epilepsy?

- **Hypothesis:**
  - *In vivo* antagomir inhibition of miR-218-5p and miR-324-5p will regulate seizure susceptibility and affect electrographic dynamics in *Cntnap2* knockout mouse model of epilepsy.

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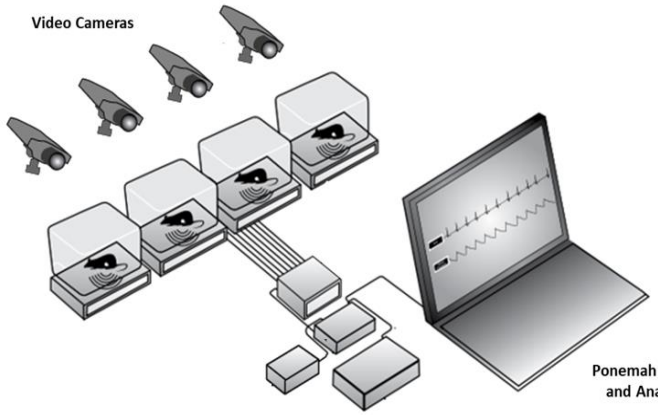
Methods

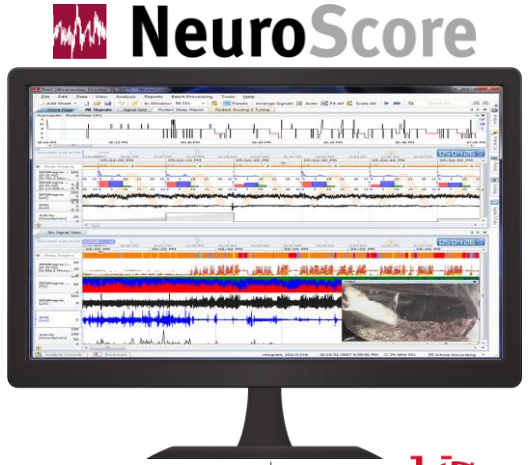
- **Mice**
  - Mice were first genotyped
  - They were then classified by gender and age
    - Both male and female mice were used at two age points
    - Age points: younger (4-6 months) or older (12-16 months)
  - This allowed us to explore age and gender specific seizure development

- **Transmitter Implantation**
  - Electrodes were surgically implanted in mice using a wireless telemetric device, and video EEG was monitored

Electrode Implantation



EEG Recording



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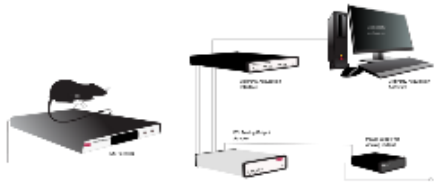
Antagomir Injection

- Antagomirs for miR-324-5p, miR-218-5p, or scrambled (control) were injected intracerebroventricularly by (ICV), and electrodes were implanted in mice in order to obtain a baseline cortical EEG measurement in young and old mice



EEG Analysis

NeuroScore

Days 3-10 (1 week):
EEG recording and video monitoring

Day 0:
ICV antagomir injection and electrode implantation

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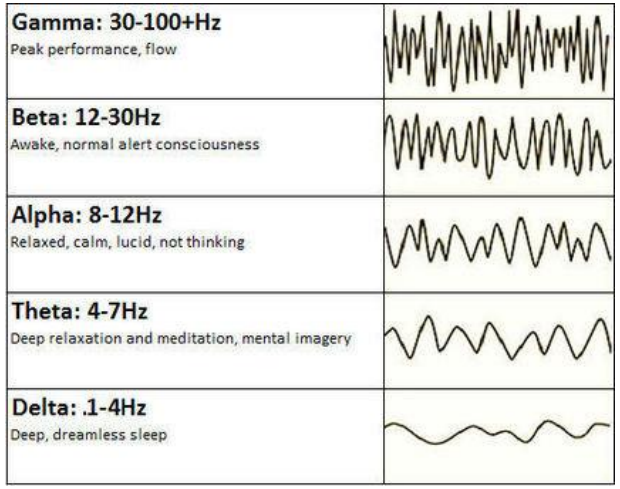
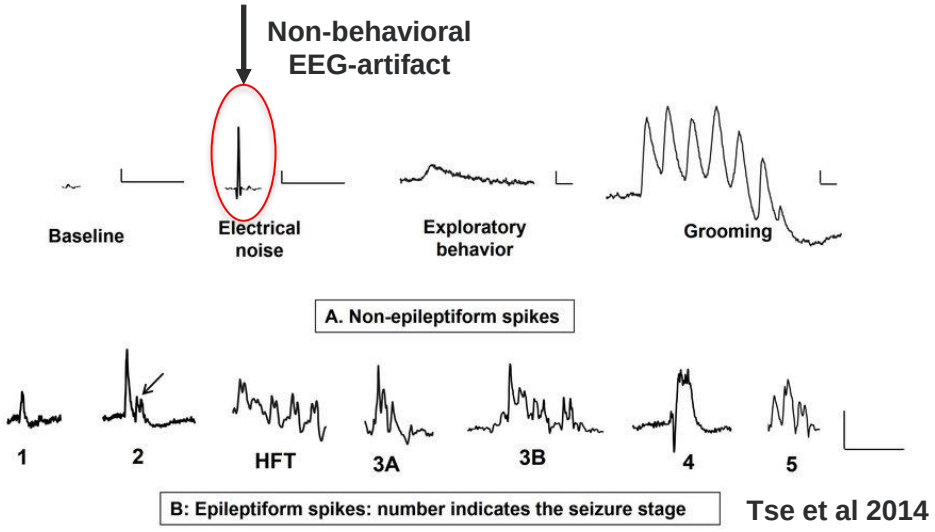
EEG Analysis

- EEG was recorded using a DSI wireless telemetry system and analyzed using Neuroscore software

- Artifacts were manually excluded from analysis **(A, electrical noise)**
- Seizures were manually scored using video EEG monitoring **(B)**

- EEG waveform voltage was recorded, and EEG power analysis was conducted after data was exported in 10 second epochs



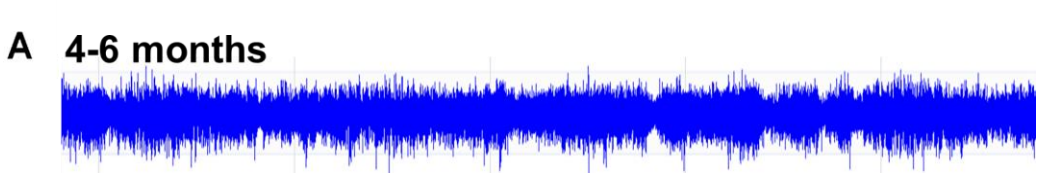


Tse et al 2014

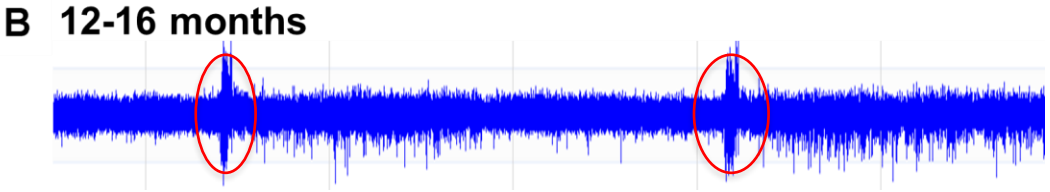
Results

Spontaneous recurring seizure (SRS) onset in *Cntnap2* KO mice

EEG and video were monitored in younger and older mice to **confirm the onset of seizures**



A: In younger mice, spikes were observed in EEG but no seizures



B: In older mice, SRS were observed (**circled**)

Seizures occur at an older age in *Cntnap2* KO mice (12-16 months)

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Effect of antagomir treatments on seizure frequency and duration in older *Cntnap2* KO mice

EEG Analysis

NeuroScore

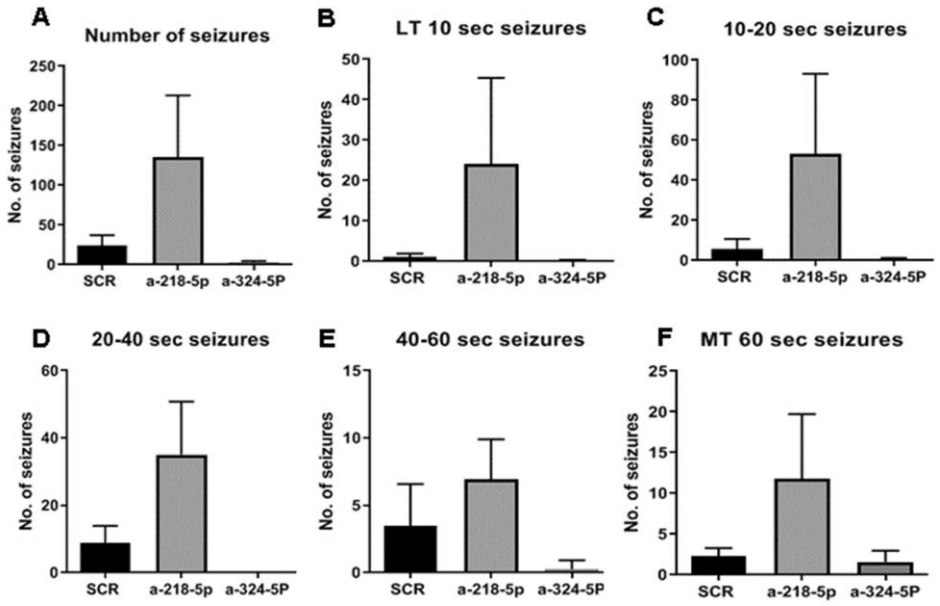
Day 0:
ICV antagomir injection and electrode implantation

Days 3-10 (1 week):
EEG recording and video monitoring

One way ANOVA (Graph pad)

a-miR-SCR: 7
a-miR-218-5p: 5
a-miR-324-5p: 8

A: p=.0184
B: p=0.438
C: p=0.354
D: p=0.109
E: p=0.229
F: p=0.366



- **Seizures were manually scored and classified based on their duration (A-F)**

miR-324-5p inhibition decreased seizure frequency and miR-218-5p increased seizure frequency across all seizure duration groups

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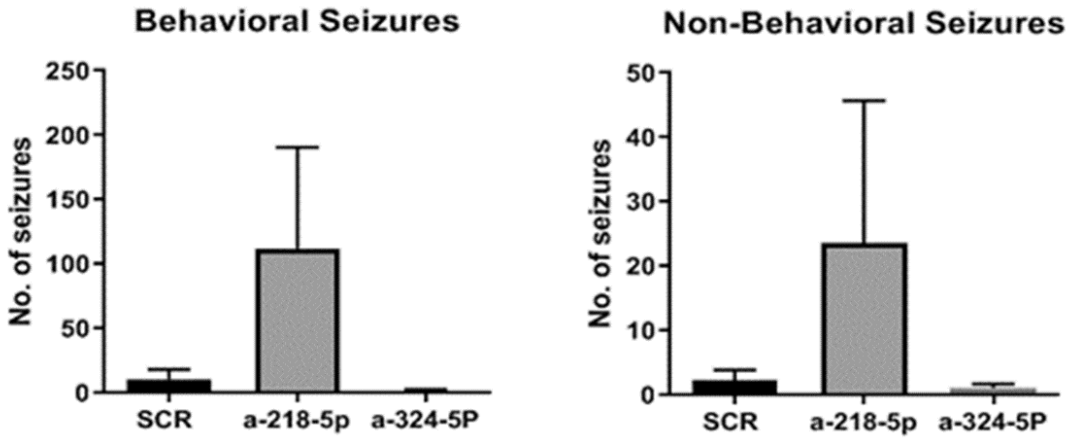
Effect of antagomir treatment on behavioral and non-behavioral seizure frequency in older *Cntnap2* KO mice



- **Seizures were also classified based on whether they were behavioral (confirmed with video) or non-behavioral (electrographic with no behavior)**

One way ANOVA (Graph pad)

a-miR-SCR: 7
a-miR-218-5p: 5
a-miR-324-5p: 8
Behavioral: p=0.298
Non-behavioral: p=0.298

Again, a-miR-324-5p treatment reduced seizure frequency and a-miR-218-5p treatment increased frequency for both groups

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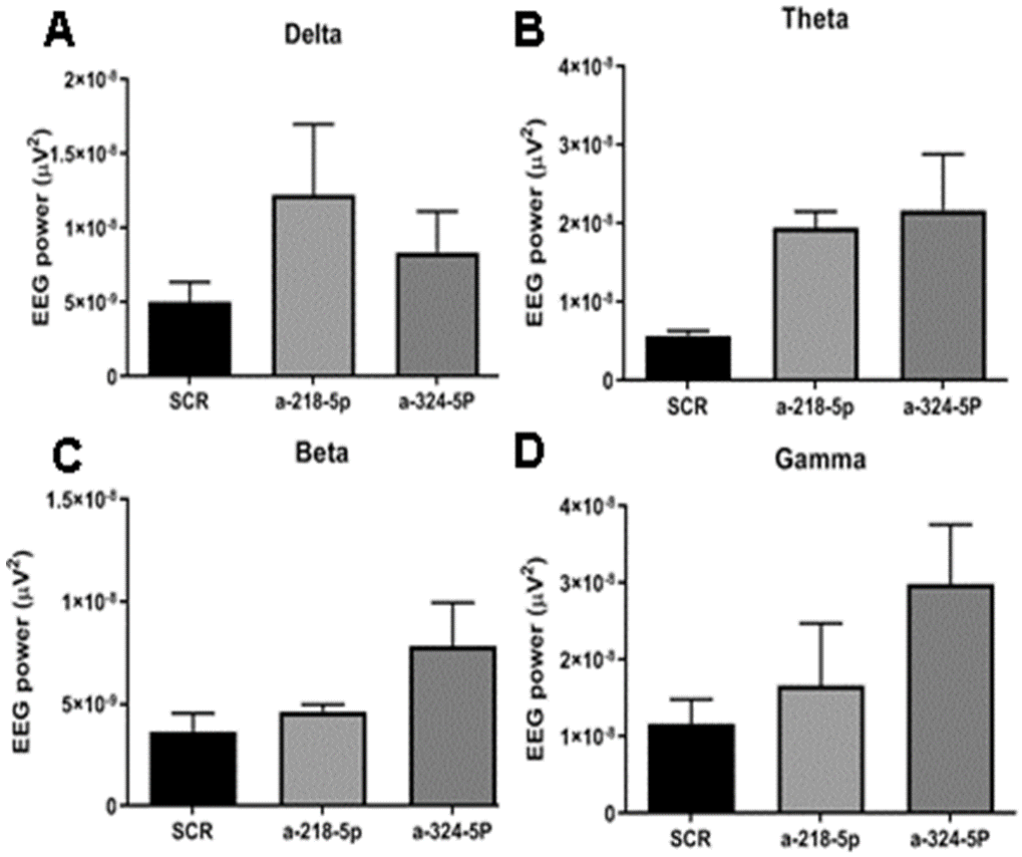
Effect of antagomir treatments on EEG power in older *Cntnap2* KO mice



- EEG recording was exported and analyzed on Neuroscore
- Average EEG power of four different waveforms, **(δ(A), θ(B), β(C), and γ(D),** were analyzed and compared between treatment groups

One way ANOVA (Graph pad)

a-miR-SCR: 3
a-miR-218-5p: 2
a-miR-324-5p: 3
A: p=0.301
B: p=0.157
C: p=0.250
D: p=0.725

No significant differences in EEG power for any waveform were found between the treatment groups

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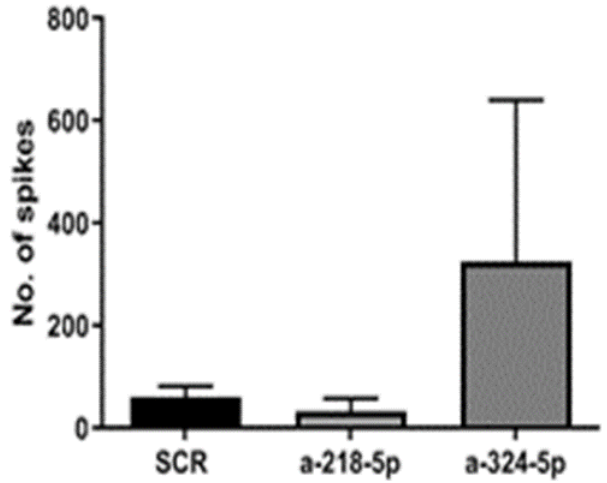
Effect of antagomir treatments on epileptiform non-seizure EEG spikes in older *Cntnap2* KO mice



- Spike analysis was conducted to determine how many epileptiform non-seizure related spikes were found in the EEG
- Spikes indicate an increase in hyperactivity in the neuronal network

One way ANOVA (Graph pad)

a-miR-SCR: 3
a-miR-218-5p: 2
a-miR-324-5p: 3

p=0.469

miR-324-5p inhibition had a trend to increase epileptiform EEG spikes

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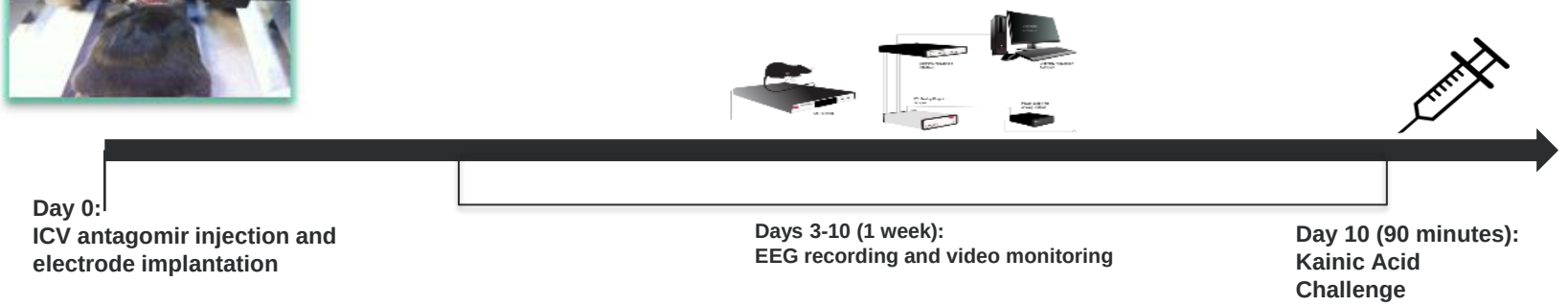
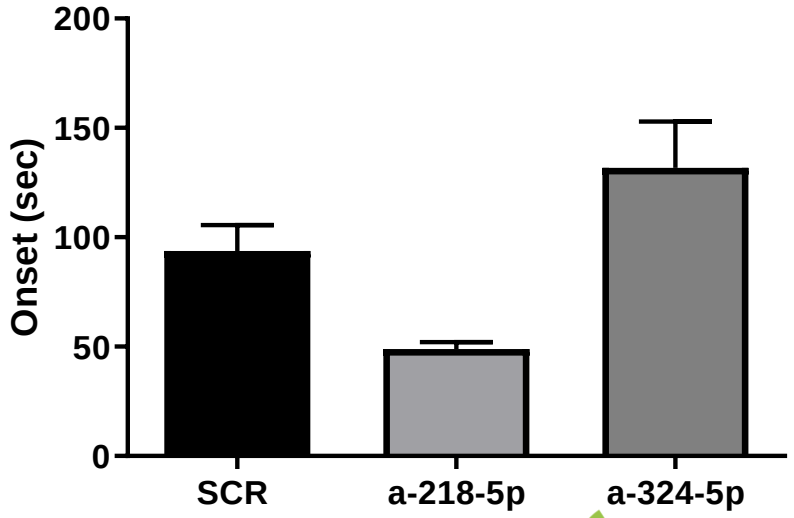
Effect of antagomir treatment on kainic acid-induced seizure onset latency in younger *Cntnap2* KO mice



- Young mice underwent kainic acid treatment after 10 days to induce seizures
- Seizure onset time was measured and compared between groups
- **Antagomir treatment for miR-324-5p significantly increases latency to KA-seizure onset while it is decreased with treatment for miR-218-5p**



One way ANOVA (Graph Pad)
a-miR-SCR: 4
a-miR-218-5p: 4
a-miR-324-5p: 5

p=0.012

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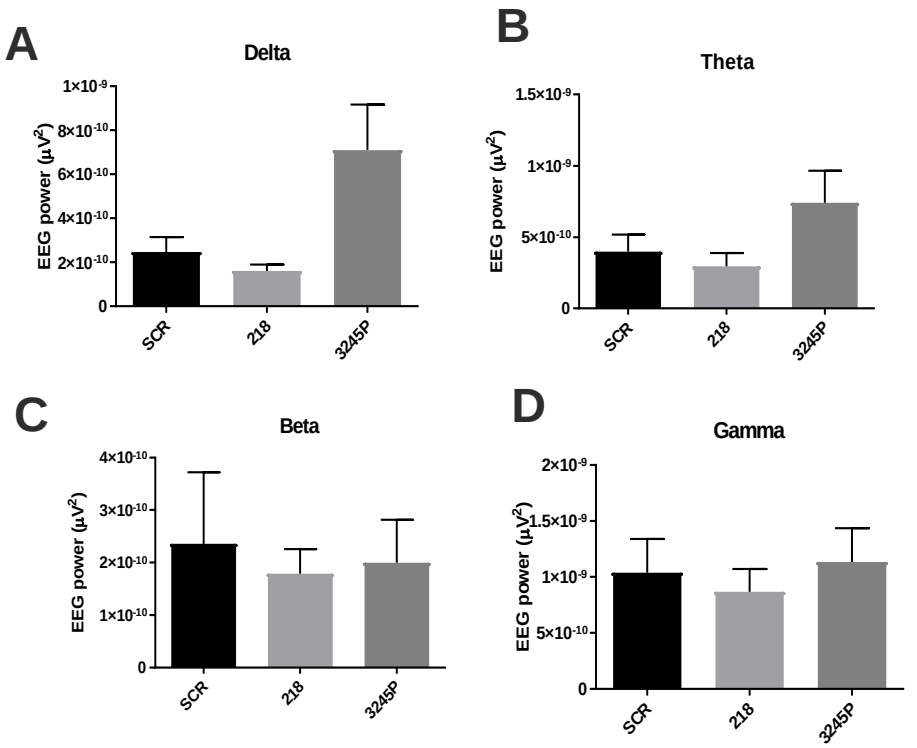
Effect of antagomir treatments on EEG power of younger *Cntnap2* KO mice

- Power analysis, following the same procedure as with older mice, was performed to assess EEG power for:
  - A: Delta wave
  - B: Theta wave
  - C: Beta wave
  - D: Gamma wave



One way ANOVA (Graph Pad
a-miR-SCR = 2
a-miR-218-5p = 4
a-miR-324-5p = 4

A trend towards significance (p=.0594) was observed of the average EEG power of Delta waves in a-miR-324-5p mice compared to other treatments, but not in any other waveforms

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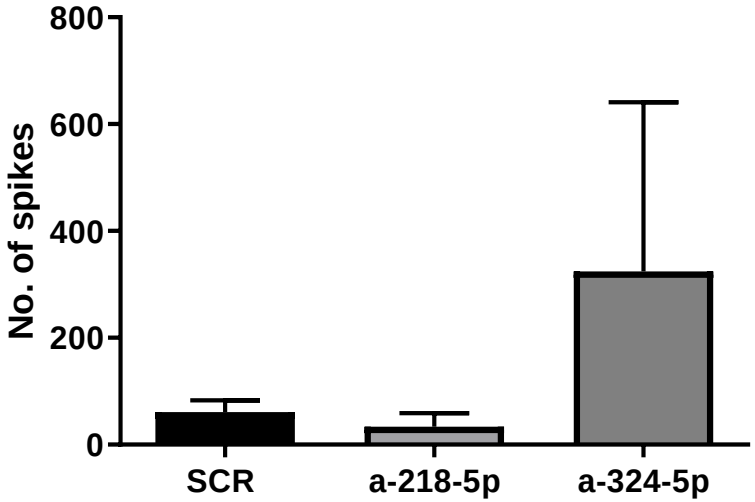
Effect of antagomir treatment on spikes of younger *Cntnap2* KO mice

One way ANOVA
a-miR-SCR: 3
a-miR-218-5p: 2
a-miR-324-5p: 2

p=0.428



- Spike analysis, following the same procedure as with older mice, was performed on younger mice
- This was another way for us to explore hyperactivity of the neuronal network

A trend was observed for an increase in spikes in mice treated with a-miR-324-5p that can be further investigated

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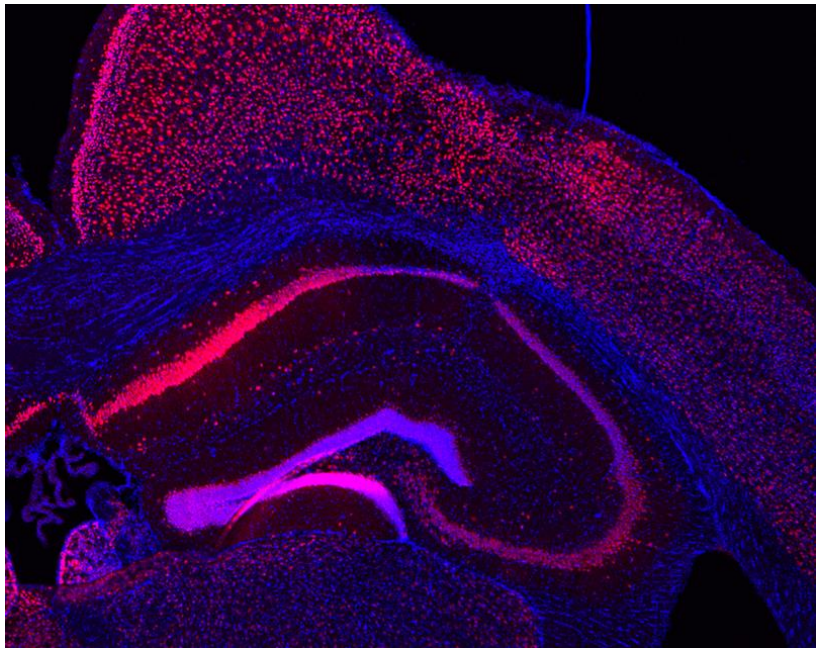
Ongoing Tissue Analysis

- Mouse brains were collected to analyze the effect of treatment on **cell death, neuronal migration** and **gliosis** in the brain

- **Ongoing immunostaining:**
  - NeuN (neuronal, A)
  - GFAP (Astrocytes)



Representative NeuN Stain

Conclusions

- **ICV injection of antagomir for miR-324-5p in *Cntnap2* KO mice resulted in a delayed onset of seizures (younger mice) as well a decrease in seizure severity (older mice)**

- ***Cntnap2* KO mice treated with miR-218-5p antagomir injection displayed an increase in seizure severity and decrease in seizure onset latency**

- **Preliminary EEG waveform power and spike analyses did not find any significant differences between EEG power and epileptiform spikes between treatment groups.**

These results suggest that both miR-324-5p and miR-218-5p differentially regulate seizures in *Cntnap2* KO mice and could potentially be important for studying the mechanism of epilepsy

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Future Aims

- **Increase sample size for EEG power analysis of younger and older mice**
- **Explore localization of effects by conducting immunohistochemical tissue analysis of hippocampus and cortex**
- **Expand EEG power and spike analysis to include sleep-wake patterns in EEG recordings**

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Thank You!

If you have any questions, feel free to contact me at: mukherrv@mail.uc.edu!