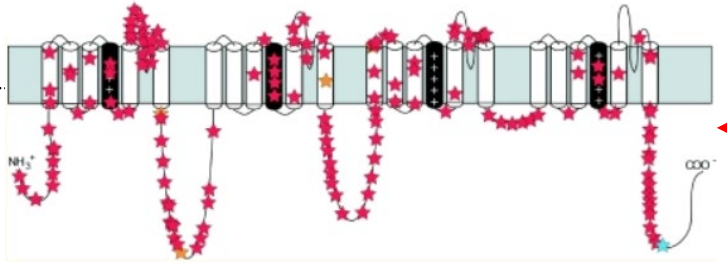
The Effects of Melatonin and DSIP on Sleep and Epilepsy in a Mouse Model of Dravet Syndrome

Mariam Benazouz & Ai Vy Nguyen
Neurological Surgery Summer Student Program
Kalume Lab

Seattle Children's
HOSPITAL • RESEARCH • FOUNDATION

UW Medicine
SCHOOL OF MEDICINE

Dravet Syndrome (DS)



Mutations in $Na_v1.1$ channel (Catterall *et al. J. Physiology*, 2010)

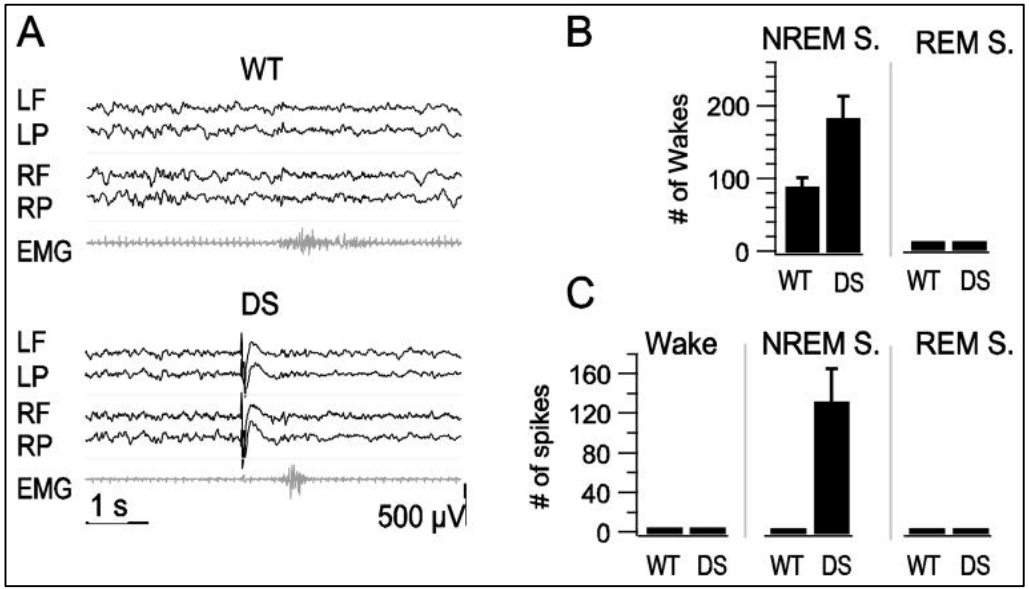
- Childhood-onset epilepsy (six-nine months old)
- Begins with seizures induced by fever or hyperthermia
- Multiple seizures of different types and levels of severity (myoclonic, tonic-clonic, absence, and partial seizures)
- Prevalence: 1 in 22,000 to 40,000 individuals
- Caused by loss-of-function mutations in the ***SCN1A*** gene (most frequent mutations in epilepsy)
- *SCN1A* is the gene that codes for the voltage-gated sodium channel, $Na_v1.1$

Why Sleep?

- Sleep disturbances are common in people with epilepsy
- Are associated with poor seizure control
- Poor quality of life of the patients
- In children with epilepsy, they can negatively affect the coping mechanism of the family.
- DS patients have severe sleep abnormalities (75% of 57 patients)
  - Abnormal sleep-wake cycle (35%)
  - Difficulty falling and staying asleep (39%)
  - Nocturnal seizures (53%)
- Poor sleep can lead to behavioral and attentional problems.
  - These can contribute to social and academic difficulties.

Licheni *et al., Developmental Medicine & Child Neurology,* 2017
Kalume *et al., Neurobiology of Disease,* 2015

Fragmented Sleep in DS Mice



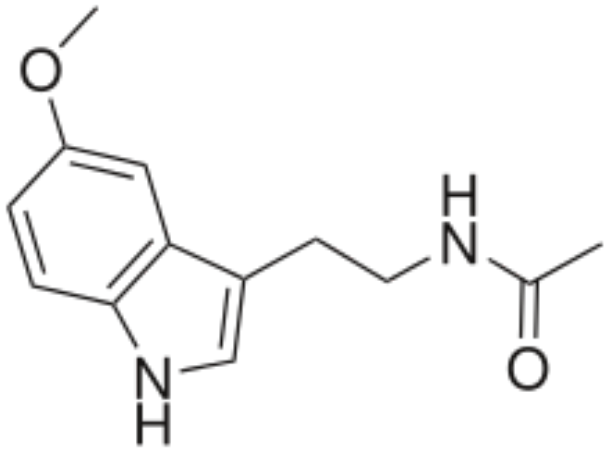
Kalume *et al., Neurobiology of Disease,* 2015

Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

Melatonin

- A natural-occurring hormone that is produced in the brain- specifically in the pineal gland- that is linked with sleep.
- More melatonin in the human body is released during the nighttime to help induce sleep
- Dosage for DS mice: 10 mg/kg

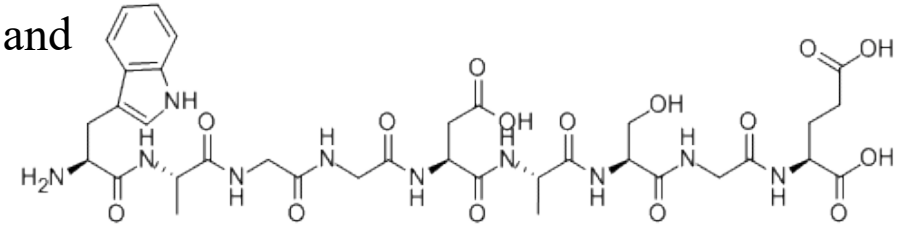


Structure of Melatonin

Licheni *et al., Developmental Medicine & Child Neurology,* 2017

Delta Sleep Inducing Peptide (DSIP)

- Member of natural sleep peptide family
- Link between DSIP and sleep is weak due to lack of isolation of DSIP gene, protein, and related receptor.
- Based on name: Inducing delta sleep (NREM)
- Dosage for DS mice: 2 mg/kg



Structure of DSIP

Graf *et al., Neuroscience & Biobehavioral Reviews,* 1984.
Kovalzon *et al., Journal of Neurochemistry,* 2006.

Aims and Hypothesis

- Reciprocal relationship between epilepsy and sleep
  - Having epilepsy may lead to poor sleep
  - Poor sleep can worsen seizures
- Aim: Evaluate the effects of melatonin and DSIP on sleep and epilepsy in DS mice.
- We predicted that melatonin and DSIP will:
  - respectively increase the temperature threshold for seizures
  - provide better quality of sleep in mice with Dravet

Methods

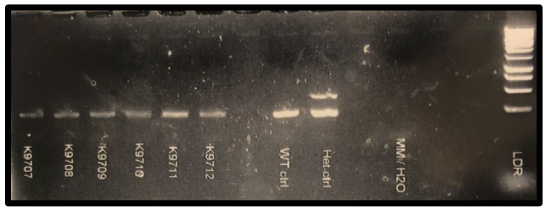
Experiment Part 1: Effects of compounds on **sleep quality**

Breeding

Het Scn1a x WT
Strain: C57BL/6
Het Scn1a mouse will experience similar symptoms to DS patients
n=6

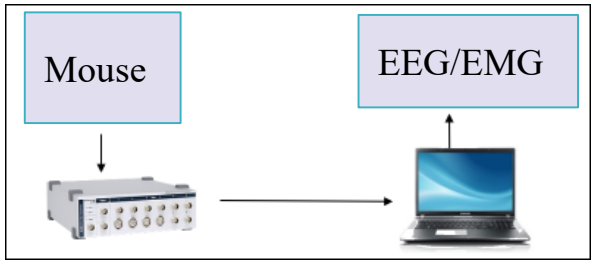
Genotyping

Confirm the right genotype for our experiments using PCR



Injection (vehicle or melatonin/DSIP, i.p.)

Vehicle: 0.3%
Tween 80 in saline



Bard, 2018

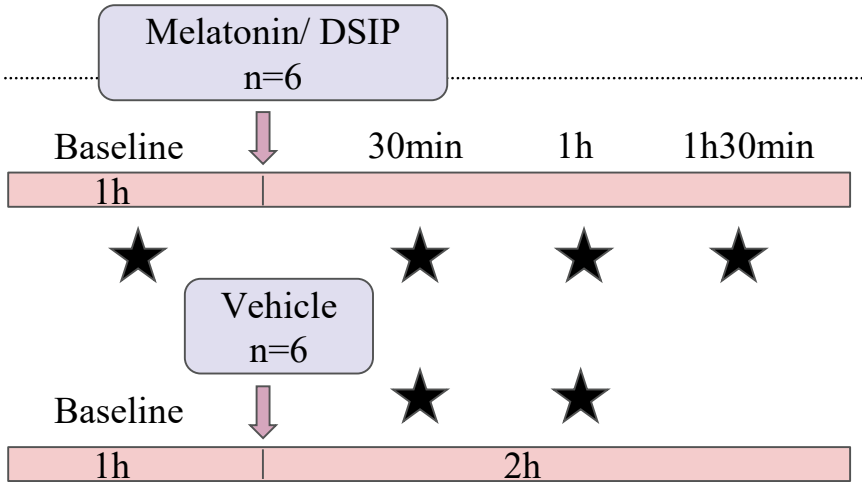
Experiment Part 2: Effects of compounds on **seizure threshold**

- DS mice also experience seizure from high temperature, just like DS patients.
- Insert probe in mice to record body temperature and let acclimatize for 5 minutes
- To induce seizure, raise temperature (0.5℃ every 2 minutes) until seizure occurs or body temperature reaches 42℃

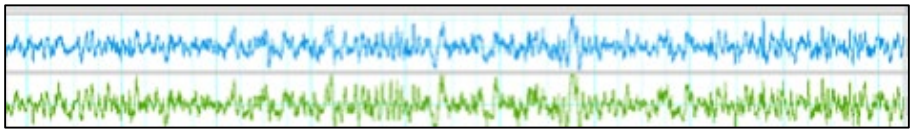
Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

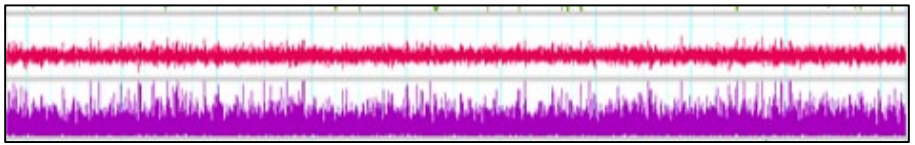
Part 1 Analysis



1. Longitudinal analysis (EEG)

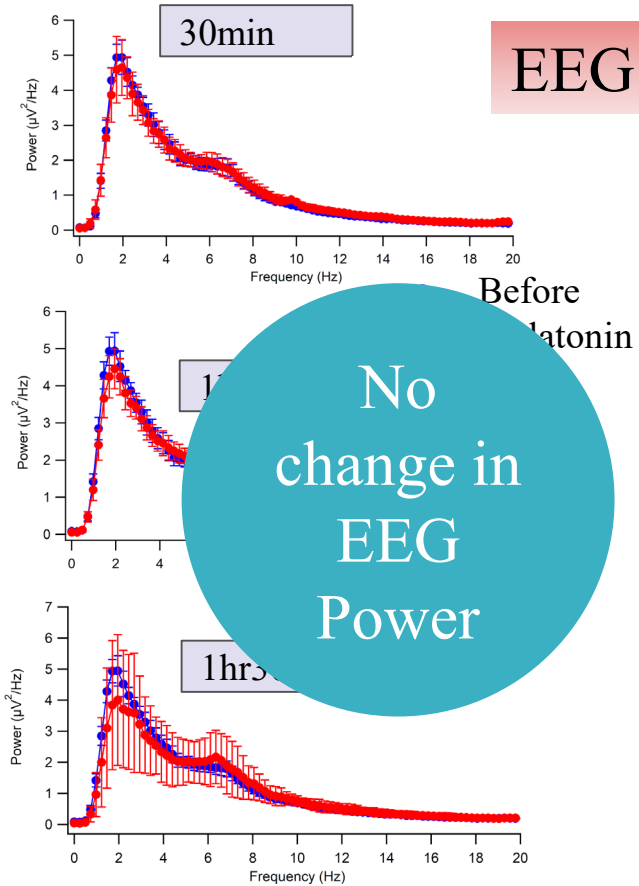


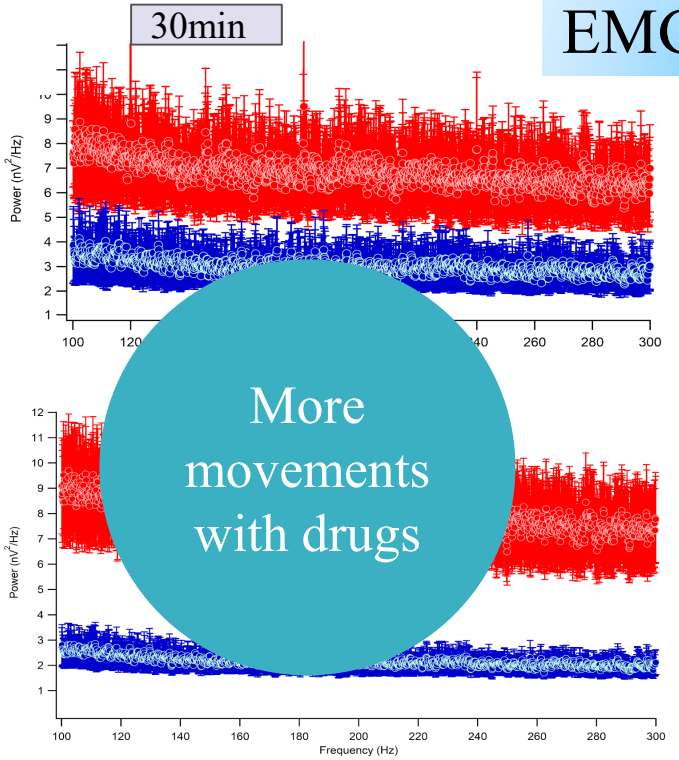
2. Comparative analysis (EMG)



Examples of EEG/EMG recording

Power Spectrum: Melatonin

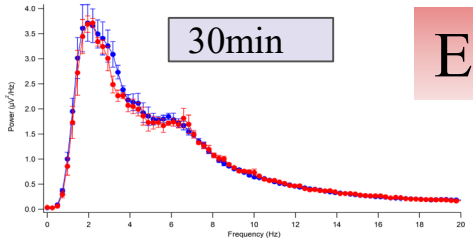


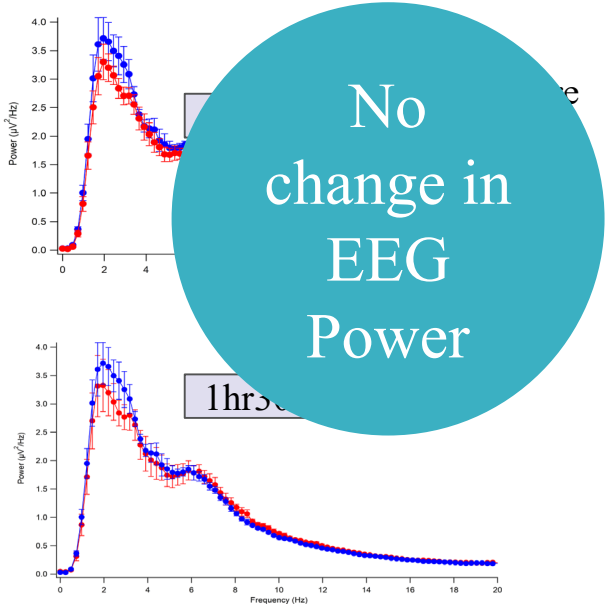


Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

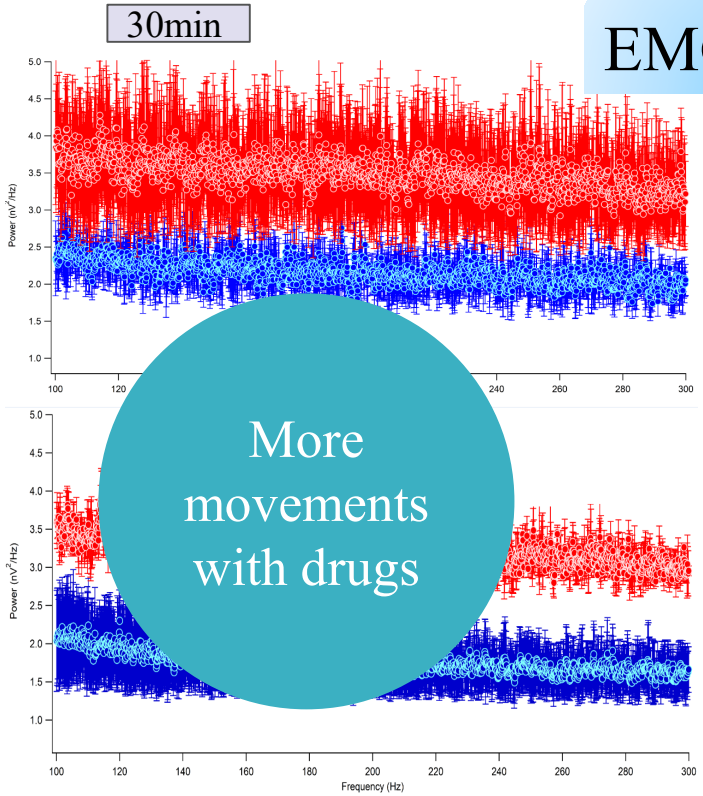
Power Spectrum: DSIP





No change in EEG Power

- Vehicle
- DSIP



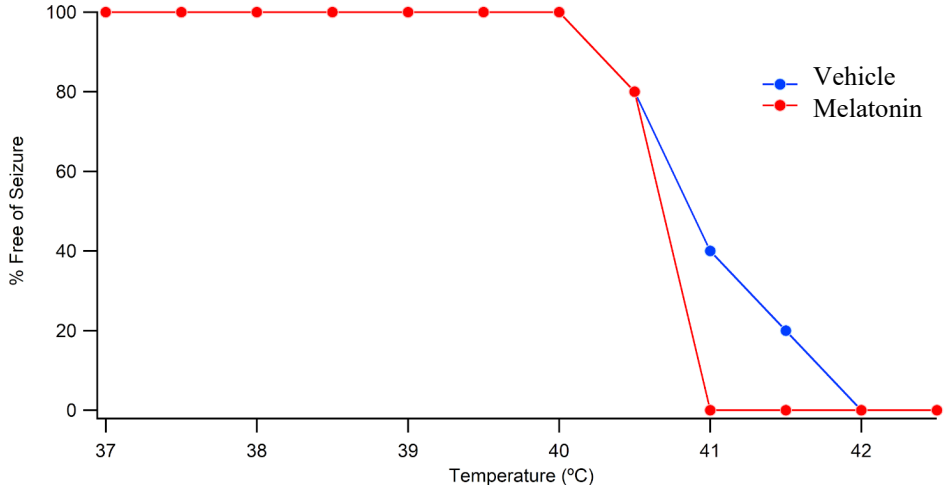
More movements with drugs

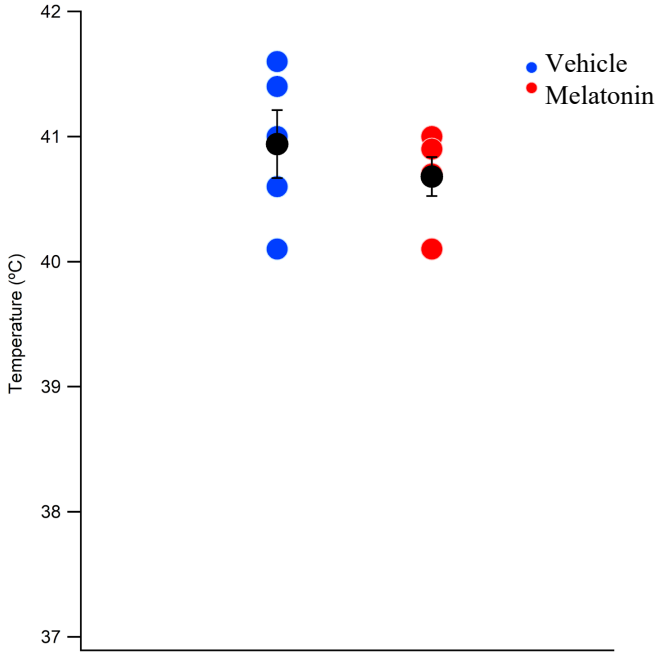
Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

Part 2: Temperature-induced seizure

Between Vehicle and melatonin

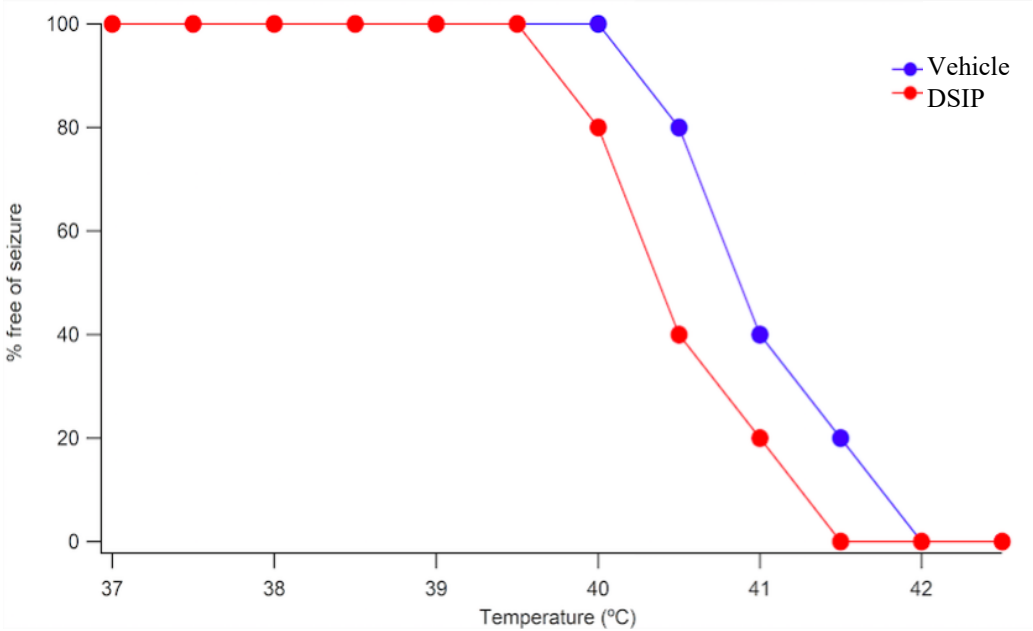


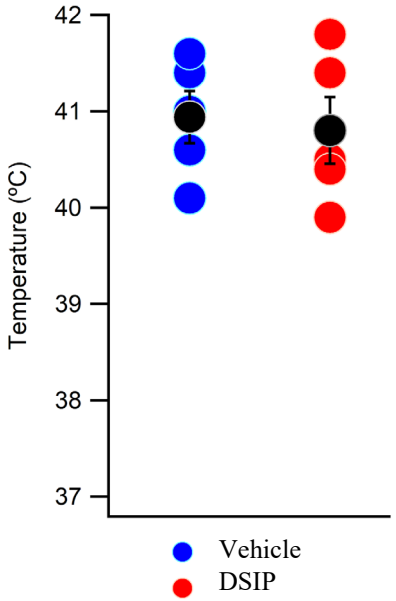


Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

Between Vehicle and DSIP





Seattle Children's HOSPITAL • RESEARCH • FOUNDATION

UW Medicine SCHOOL OF MEDICINE

Conclusion

- Melatonin (10mg/kg) and DSIP (2mg/kg) did not exhibit any effect on EEG power density.

- Melatonin/DSIP did not affect the susceptibility to thermally induced seizures at the screened doses.

Future Directions

- Test different dose of melatonin and DSIP
- Increase the number of animals
- Osmotic Pump - for extended drug release

Azlet Osmotic Pump

Acknowledgments

Dr. Richard Ellenbogen and Mrs. Sandra Ellenbogen

Jana Pettit

Jim Pridgeon

Dr. Christine Mac Donald

Ellie Thorstad

UW Neurological Surgery Donors, Faculty, Staff, and Residents

Dr. Franck Kalume

Dr. Rufi Dalvi

Michelle Bard

Other interns in Kalume Lab

Grant: NIH NINDS R25NS095377 - Summer Research Experience in Translational Neuroscience and Neurological Surgery